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FIRST ANNUAL MEETING OF THE INDIAN ACADEMY OF SCIENCES.

(Held at Bombay on 18th December 1935.)

PRESIDENTIAL ADDRESS.

By Sir C. V. Raman, Kt., F.R.S., N.L.

The Indian Academy of Sciences was registered at Bangalore on the 24th of April 1934, and was formally inaugurated at a public meeting held at the Indian Institute of Science on the 31st July 1934, by Amin-ul-Mulk Sir Mirza M. Ismail, Dewan of Mysore. In the ordinary course of events, the Annual Meeting should have been held in July last. But for various reasons it was felt desirable to postpone it till the cold weather so that it would be possible for the Meeting to be conveniently held at some centre outside Bangalore where our Fellows could assemble. There is a peculiar appropriateness in that the Academy which was inaugurated at Bangalore should hold its first annual meeting at Bombay. It is known to all of you that if Bangalore to-day occupies a significant position in the world of science, it is to no small extent due to the far-sighted generosity of a great and enterprising citizen of Bombay, the late Mr. Jamsetjee Nusserwanjee Tata. At a time when the Universities of India were few in number and were purely examining bodies, Mr. Tata conceived the idea of creating an Imperial Teaching and Research University for the whole of India which would have aims and ideals approximating to those of such ancient foundations of learning as Oxford and Cambridge. In Mr. Harris's life of Tata, we have an authoritative account of the early history of Mr. Tata's scheme. We read that the two reasons which finally lead to Bangalore being the recipient of his princely benefaction to the cause of learning were firstly, the agreeable climate of Bangalore which he regarded as particularly suitable for a centre of advanced teaching and research, and secondly, the generous offer of the Maharaja of Mysore of half a square mile of land, five lakhs of rupees as a capital grant and an annual subsidy of one lakh of rupees towards the establishment of the Institute at Bangalore. Forty years ago, in this way was forged an intellectual link between Bangalore and Bombay which is now indissoluble and which has had and will, I believe, continue to have a far-reaching influence on the progress of science in India.

The idea of establishing an All-India Academy of Sciences was first clearly put forth in an editorial article in the Journal "Current Science"

published from Bangalore in May 1933. After a period of discussion and consultation with scientific men all over India, the decision to inaugurate the Academy with its provisional headquarters at Bangalore was finally taken in April 1934. I shall, in the course of this address, refer more in detail to the progress which has since been achieved by the Academy and which has amply justified that decision. But even at this early stage, it is appropriate to mention the large part which our friends in Bombay have played in achieving that progress. At the present time, no fewer than thirty-eight of our Fellows are resident in the Bombay Presidency. The election of such a large number to the distinction of the fellowship has been a natural consequence of the remarkable development in recent years of scientific research activities in the Bombay Presidency. I shall have more to say about this later in my address, and it will be sufficient to remark now that the published Proceedings of the Academy bear ample witness to the scientific energy of Dr. T. S. Wheeler and his colleagues at the Royal Institute of Science and to the deep interest taken in the progress of the Academy by the officers of the Meteorological Department at Poona, and the schools of scientific research which have developed at Poona and other parts of the Bombay Presidency. For all these reasons, it is very appropriate that we meet to-day at Bombay. I hope that this gathering will be the first of a series to be held year after year at various centres of scientific research in India. It is desirable that the annual meetings of the Academy should serve to bring together its Fellows from various parts of India at least once a year and thus to strengthen the feeling of scientific comradeship that unites them. Science like other products of human activity, stands to gain immensely from the personal contacts of leading workers. It is earnestly to be hoped that our present meeting will furnish opportunities for such contacts and thus serve to promote the cause of the advancement of science in our great country.

Before I pass to review the work and progress of the Academy since its foundation, I must express the gratitude of the Council to Your Excellency in having consented to grace the occasion to-day and encourage us by your presence here. I must also express the gratitude of the Council to the Fellows of the Academy in Bombay headed by Dr. Wheeler our Vice-President, and to the Reception Committee presided over by you Mr. Vice-Chancellor, who have been at immense pains to organise this our first Annual Meeting on a scale worthy of the occasion.

When the Academy was inaugurated, it commenced its activities with 65 Fellows. The Council obtained permission from the General Body of Fellows to elect fresh Fellows up to a maximum of 200 and also Honorary

Fellows up to a maximum of 30 from amongst the most distinguished scientists of the world. This permission has been acted upon and we have to-day 173 Fellows in India and 30 Honorary Fellows. Our Honorary Fellows include some of the most active and influential scientific men in Europe and America, whose sympathy and co-operation will, I am sure, be of the greatest benefit to the Academy.

The British list of Honorary Fellows includes Lord Rutherford, Sir William Bragg, Sir F. Gowland Hopkins, Sir John Russell, Prof. O. W. Richardson, Prof. Robert Robinson, Prof. A. V. Hill, Prof. P. A. M. Dirac, Prof. A. C. Seward and Prof. G. H. Hardy. The American list includes Prof. R. A. Millikan, Prof. A. H. Compton, Prof. N. L. Bowen, Prof. Harvey Cushing, Prof. D. D. Van Slyke and Prof. G. N. Lewis. The German list includes Prof. A. Sommerfeld, Prof. W. Heisenberg, Prof. Hans Fischer, Prof. H. Wieland and Prof. F. Paschen. From France we have Prof. A. Cotton and Madame Irene Curie-Joliot. From Sweden we have Prof. K. M. G. Siegbahn and Prof. Th. Svedberg. From Denmark and Holland we have respectively Prof. Niels Bohr and Prof. P. Zeeman. From Italy we have Prof. E. Fermi and Prof. S. Belfanti and from Russia Prof. I. P. Pavlov.

It is noteworthy that the list of 30 includes one woman scientist, Madame Irene Curie-Joliot. It must have given our Fellows great pleasure to read the recent announcement of the award of the Nobel Prize in Chemistry to this lady and her husband jointly.

Our list of Fellows in India is representative of every important branch of science. Physics and Meteorology are represented by 34 Fellows, Mathematics and Astronomy by 18, Chemistry by 40, Zoology and Anthropology by 17, Agriculture, Forestry and Botany by 35, Medicine by 15 and Geology by 8. We have only 6 Engineering Fellows but they include some very distinguished names including some very familiar in Bombay, *viz.*, Sir M. Visvesvaraya and Dewan Bahadur N. N. Iyengar. Our list of Fellows is also representative of all parts of India. Bombay heads the list with 38 Fellows, closely followed by the Madras Presidency by 35 and Mysore State by 33. Other provinces are also well represented. We have 21 Fellows in the United Provinces ; 13 from the Punjab, 11 from Bengal, 8 from the Central Provinces ; Bihar and Orissa, Hyderabad, Travancore and Burma are also represented in our list.

The scientific activities of the Academy may be considered under the three heads :—

Meetings for discussion of research papers.
Symposia on special subjects.
Publication of the Proceedings.

I shall consider the last first because in a country like India separated by great distances, by far the most important service that can be rendered to science by the Academy is the regular issue of a scientific journal of high standing in which scientific papers of its Fellows can find prompt publication. I think it will be generally agreed that the Academy has achieved very gratifying success in this direction. Ever since the formal inauguration of the Academy, the Proceedings have appeared month after month with unbroken regularity on the due date. A very great amount of material has reached the Academy from many quarters. The examination of this material and the selection of suitable papers has naturally been a formidable task. That it has so far been accomplished without any signs of breakdown is largely due to the co-operation which the Academy has been so fortunate to secure. A special word of praise is due to the Superintendent of the Bangalore Press who has maintained a high standard of printing both as regards accuracy and technical finish and has enabled the Journal to appear with unfailing punctuality. To the numerous Fellows who have acted as referees for papers often at great cost of time and trouble, the Council are deeply indebted. A heavy burden has also fallen on the Secretaries and on the Manager of the office which they have discharged with conspicuous devotion and success. I believe our Fellows have by this time learnt to look forward to the appearance of the Proceedings on the first of every month and to peruse its contents with eagerness and satisfaction. The volume of published material has grown so rapidly that commencing from July 1935 it was found necessary to separate the Proceedings into two parts, A, Physical and Mathematical Series, and B, Biological Series respectively. The two numbers of the Journal appearing in each month have each contained roughly 100 pages of printed matter together with a very substantial number of illustrated plates. In view of the volume of published matter, the Council have decided in future to issue two volumes instead of one per annum for each of the two sections of the Proceedings.

I will next refer to the Symposia organised by the Academy. There was one in August 1934 on Molecular Spectra which was attended by 50 Fellows from all over India. The shorter papers submitted for this symposium have all been published in the Proceedings. A very valuable and detailed report by Prof. R. Samuel of the Aligarh University has already been printed and circulated to leading specialists on the subject. A detailed report by Mr. N. S. Nagendra Nath on the subject of Dynamics of Molecular Vibrations is also to be printed and issued shortly together with Dr. Samuel's Report as a special publication. In October 1935,

a symposium on Disease Resistance in Plants was held at Coimbatore. This was largely attended and was a successful gathering, mainly as the result of the efforts of our Coimbatore friends who worked hard to organise the function. At the present meeting in Bombay, a symposium on Colloid Science has also been arranged.

An important part of the regular work of the Academy is the holding of scientific meetings at which papers presented to it are read and discussed. While such meetings are usually held at Bangalore, the Council have also encouraged the idea of meetings being held at other centres for the reading of papers on the occasion of special gatherings such as Symposia and the annual meetings.

A matter of great concern to the Academy is the question of providing money for these activities, especially for the cost of publication of the Proceedings which is very heavy. That it has been possible at all to carry on the work of the Academy without a complete financial breakdown is largely due to the generosity of the external authorities who have come forward to help us. Chief amongst these, I should mention the Government of His Highness the Maharaja of Mysore who have sanctioned a grant of Rs. 3,000 per annum for a period of five years. His Highness the Ruler of Bhopal has been pleased to sanction an annual recurring grant of Rs. 500, and the Government of His Highness the Maharaja of Cochin have also sanctioned an annual recurring grant of Rs. 250. The Imperial Council of Agricultural Research have sanctioned a grant of Rs. 500 per annum for 3 years. The latest benefaction to the Academy is from the Government of His Highness the Maharaja of Travancore of Rs. 1,000 for this year. The Council of the Indian Institute of Science sanctioned a grant of Rs. 2,000 for the current year. The University of Nagpur have given us Rs. 100 and one of our Fellows, Mr. T. W. Barnard, has made a special contribution of Rs. 50.

It must be obvious that the publication of a scientific journal rather, of two scientific journals appearing month after month, is a very expensive proposition. Unless we have an assured income of at least Rs. 25,000 per annum, it will not be possible to carry on this work in a satisfactory manner. Only about one-third of this sum can be found from the regular subscriptions of our Fellows. In these days, the building up of a subscription list for a new scientific periodical is a slow and difficult business. It is here, however, that great assistance can be rendered to us by the educated public in India. If every college, every scientific institution and every department of the local Governments subscribed, as it should, for one copy of the Proceedings of the Academy, our financial problem would be greatly eased. I earnestly appeal to all the other Governments and Universities in India to

come to our aid. Even a modest annual contribution from each of them would aggregate to a total sum which would enable the Academy to go forward in its great task without fear of financial breakdown.

I think it would be not inopportune to consider at this stage the nature of the services which the Academy can render to science in India. We live in an era of scientific progress and it is a very gratifying feature that India is beginning to pull its weight in this respect. Modern scientific progress shows side by side two apparently contradictory features. On the one hand, we have an enormous accumulation of raw scientific material, the significance of which, in many cases, is hardly apparent except to specialists in very limited fields of investigation. On the other hand, we have a great process of scientific synthesis going on tending towards the simplification and unification of the fundamental principles of natural knowledge in all its ramifications. It should never be overlooked that science is in reality a great impartible estate and that the boundaries drawn across it to divide it into restricted fields are in essence artificial. I think the history of science has shown over and over again that it is only by boldly cutting across these artificial boundaries that progress of real significance can be achieved. It is precisely this feature that lends importance to the activities of such an Academy as ours where men of science of widely different scientific interests come together in a common endeavour and seek to understand each other's points of view. While specialisation is necessary, an excessively narrow outlook defeats the primary purpose of science which is to advance our essential comprehension of nature as a whole. It is, therefore, one of the most important functions of our Academy to promote co-operation between men who profess knowledge of different branches of science. This is effected in various ways. In the Proceedings of the Academy the Fellows and indeed all scientific men have an opportunity of obtaining at least a general idea of what is being done in India in fields of knowledge other than their own speciality. In the scientific meetings of the Academy and especially in the Symposia, they have a valuable opportunity of discussing problems of common interest from different points of view.

I will also say a word about the Academy in relation to the nation at large. It is inevitable that the Academy, consisting as it does of the most active workers in the country who are representatives of the different parts of India and of different branches of science, will soon come to be regarded as the most authoritative body to speak in the name of India on all matters touching the progress of science. The potentialities of such an Academy in the way of national service are almost unlimited. What it can actually

achieve depends on the measure of support and recognition that it receives from the Governments of India and from the general public. I do not think that any calls for service from responsible quarters will find us unwilling or unprepared.

According to the Memorandum of Association, the headquarters of the Academy has been fixed at Bangalore for a period of three years in the first instance. I have no doubt it is the general feeling of all our Fellows that this location has fully justified itself. In this connection, I should mention the generous personal gift by His Highness the Maharaja of Mysore of ten acres of land in the vicinity of the Indian Institute of Science as a permanent location for the Academy. The location selected is a historic spot close to one of the four towers set up by Kempe Gowda, a former Hindu ruler, as a limit for the extension of his city. A relief map shows this site to be the highest spot in Bangalore. Indeed, standing on it at ground-level we see a magnificent panorama stretching out towards the horizon in all directions with Nandidroog in the blue distance towards the north, Sankey's Reservoir and the City of Bangalore to the south, the Palace Gardens to the east and the Indian Institute of Science to the west with the Sivaganga hills looming in the distance. Such a spot is indeed a worthy site for the location of an Academy of Sciences intended to play a great part in the intellectual life of the nation. Such a site also demands a noble edifice which would catch the eye and strike the imagination of both the present and future generations. Has not Bombay some far-sighted and philanthropic donor who would come forward to build an Academy of Sciences for all India and thus immortalize himself and find a place in the memory of India side by side with Jamsetji Tata. The permanent location at Bangalore of an Academy of Sciences would indeed be a fitting completion of Tata's great work. The Academy would serve as a link between the Institute and the outer world of science, each strengthening the other and helping it to reach the full fruition of its aims.

CONTRIBUTION TO THE LIFE-HISTORY OF *STELLARIA MEDIA* L.

By Prakash Chandra Joshi.
(From the Department of Botany, University of the Panjab, Lahore.)

Received December 2, 1935.
(Communicated by Dr. H. Chaudhuri.)

1. Introduction.

Stellaria media L. is a member of the sub-family Alsinoideæ of the family Caryophyllaceæ. It is a plant of very wide distribution, and is most variable in its external characters, several varieties having been recognised. In the Panjab it occurs as a small procumbent and profusely branched annual herb in waste places and also as a weed of cultivation.

Megasporogenesis of *Stellaria media* has been studied both by Gibbs (1907) and Rocén (1927), but it is seen that the results of the two contradict each other. According to the former, the sub-epidermal primary archesporial cell of the ovule develops directly into a megaspore without undergoing any cell division after the well-known "*Lilium-type*". On the other hand, the latter states that the primary archesporial cell definitely cuts off a wall cell to outside, and later a row of four or three megaspores is formed, only the lowest one of which develops into the embryo-sac in a normal manner. Gibbs in his paper has in addition described the complete development of the embryo-sac of *Stellaria media* and a few stages in the formation of the embryo. Being mostly interested in the nutritive phenomena of the embryo, she did not follow the actual sequence of its cell divisions.*

Rocén's work is quite recent, but as it extends to several families and numerous species of the order Centrospermales, it does not appear to be very intensive in respect of any single species. On the contrary, Gibbs' account, although old, is confined only to the sub-family Alsinoideæ and mostly to *Stellaria media*. It was, therefore, suggested to the writer by the late Prof. S. R. Kashyap to revise the previous work on megasporogenesis of the plant and further to study the development of its pollen-grain, regarding which nothing has been done so far.

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* The writer has undertaken to deal with full embryogeny in a separate paper.

2. *Material and Method.*

The material for the present investigation was collected from Lahore during the winter months of 1933. Flowers were fixed at different hours of the day in various fluids. Allen's modification of Bouin's fluid P.F.B.$_{15}$ (McClung, 1929) gave best results for younger stages and formalin-acetic-alcohol for older ones. After imbedding in paraffin, sections were cut 6 to 10 μ in thickness. Haidenhain's iron-alum hæmatoxylin was mostly used for staining. Orange G. in clove oil proved to be quite a good counter-stain with it. A concentrated solution of picric acid was also used for destaining instead of 2% iron-alum solution, and it served the purpose satisfactorily.

3. *Investigation.*

Microsporogenesis.—The young stamen is early differentiated into a basal filament and a terminal anther. The anther is at first a homogeneous mass of small meristematic cells covered externally by an epidermis. In its outline it is almost circular in a cross section. With continued growth it becomes oval, later bi-lobed and then faintly four-lobed. The primary archesporium is differentiated almost simultaneously with the appearance of the four lobes, and it consists of four bands of hypodermal cells, one in each lobe. Each band is four to six cells long (Fig. 2) and two cells broad (Fig. 1), but at the upper and lower ends it is generally reduced to only a single cell in width. A fairly extensive archesporium has also been reported in *Digera arvensis* (Joshi and Rao, 1934) and *Portulaca oleracea* (Cooper, 1935) from among the members of the Centrospermales so far investigated. The archesporial cells are, as usual, easily distinguishable from the rest of the cells of the anther by their larger size, larger nuclei and more densely staining cytoplasm. The anther lobes become more and more prominent after the differentiation of the primary archesporium.

The primary archesporial cells divide. The first division takes place in a periclinal direction, thus giving rise to a layer of primary wall or parietal cells to the outside and a layer of primary sporogenous cells to the inside (Fig. 3). The primary parietal cells increase in size, and undergo both periclinal and anticlinal divisions to form three to four cell layers of the parietal tissue situated in between the epidermis and the sporogenous cells of the anther (Figs. 4 and 5). The outermost parietal layer later becomes differentiated into an endothecium and the innermost layer into a tapetum. The sporogenous cells take no part in the formation of the latter. The nature of the tapetal cells is described later on.

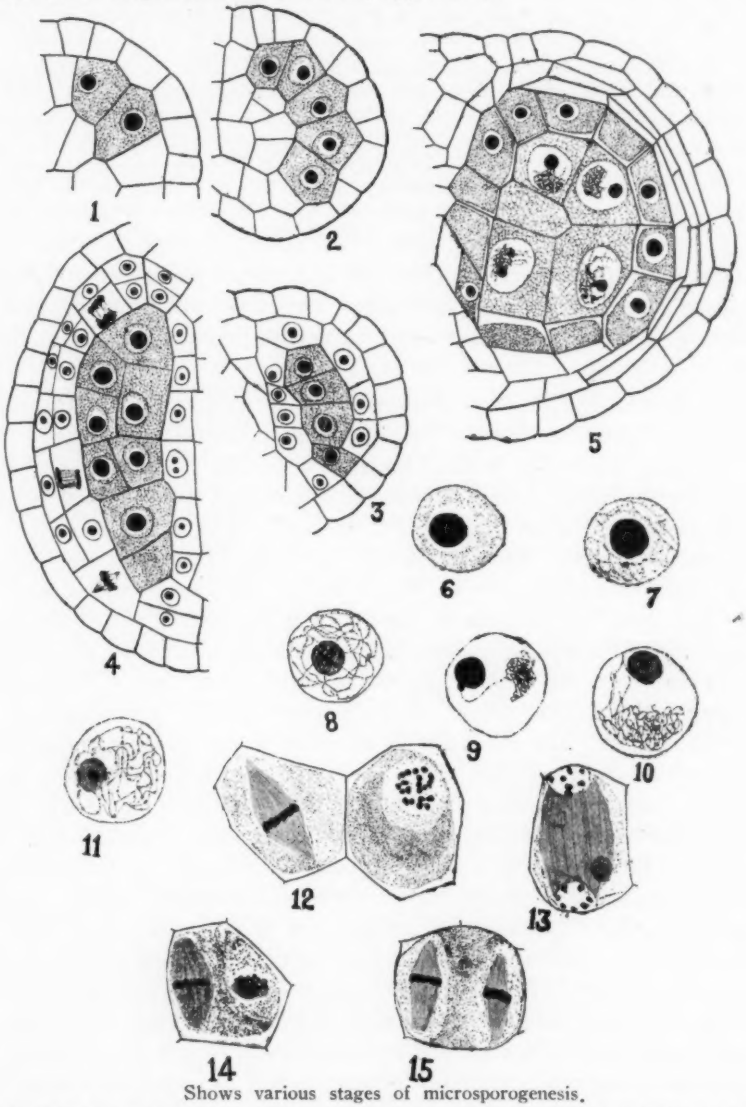
The division of the sporogenous cells occurs (Fig. 4), and as a result of this two to five (most commonly four) microspore mother-cells are seen

in a cross section of an anther lobe (Fig. 5), the number decreasing towards the two ends. All the cells divide neither simultaneously nor in the same plane. On the whole about twenty microspore mother-cells have been counted in each sporangium. Thus roughly speaking, each primary sporogenous cell undergoes only one mitotic division. As each mother-cell later on produces four microspores, the total output of pollen of a single stamen of *Stellaria media* may, therefore, be estimated to be nearly 320.

The tapetal cells show the usual characters—oblong shape in section, broad, large nuclei, richly protoplasmic contents and a deeply staining capacity. At first the cells are uninucleate but later the nuclei divide. This occurs about the time of diakinesis or a bit earlier. The division is mitotic as has been described by Cooper (1933) in many other plants. The tapetum attains its maximum development soon after the second meiotic division (Fig. 16). Occasionally three or four nuclei have been noticed in a single tapetal cell, and a single nucleus may contain two or three nucleoli. Another feature of the tapetum of *Stellaria media* is the entire lack of vacuoles. The tapetal cells, moreover, always remain at the periphery even when their inner walls become disorganised, and never move inwards to form any kind of periplasmodium.

The spore mother-cells undergo a fairly long period of rest, and during this interval the size of their cells and nuclei increases. Unlike flowering plants in general, the mother-cells neither round off themselves and separate from each other at this stage, nor do their walls become thickened. They remain packed together as is the case in *Digera* (Joshi and Rao, 1934), *Althœa*, etc. (Coulter and Chamberlain, 1903). Coulter and Chamberlain attribute this latter condition to limitation of space due to tardy disorganisation of tapetum or its failure to disorganise, but this explanation does not hold good for *Stellaria media*. In this plant at this period the anther enlarges considerably in size, and both the tapetum and spore mother-cells fail to keep pace with it. As a result of this (stretch) the walls of the tapetal cells break one after another, and a space arises in between the tapetum and the mass of sporogenous cells. The spore mother-cells, packed together, float in the cavity of the sporangium. They show the first sign of rounding at about the time of diakinesis. The spore mother-cell just before entering the first or the heterotypic meiotic division is polygonal in outline and has a thin cell wall. It is completely filled with cytoplasm which is alveolar in structure and is devoid of any deeply staining matter. In its centre it has one spherical nucleus which has got a fine nuclear membrane. Inside the nucleus there is a single, rather large, spherical nucleolus which stains intense black with hæmatoxylin (Fig. 6) The nucleolus is located either in the centre or on

Note:—All the figures are from *Stellaria media*, and have been drawn with the help of a camera lucida. Magnifications given are only approximate.



Shows various stages of microsporogenesis.

Fig. 1.—Part of t.s. of a young anther, showing two primary archesporial cells in a single anther lobe. × 900.

Fig. 2.—L.s. of a single lobe of a young anther, showing five primary archesporial cells. × 900.

Fig. 3.—L.s. of an anther lobe, showing the division of the primary archesporial cells into primary wall cells and primary sporogenous cells. × 900.

FIG. 4.—L.s. of an anther lobe, showing further development of sporogenous and wall cells. × 900.

FIG. 5.—Part of t.s. of an anther, showing 3 to 4 layers of wall cells below the epidermis, innermost modified into tapetum, and spore mother-cells in synizesis stage. × 900.

FIGS. 6–15.—Reduction division in microspore mother-cells. × 1350. Fig. 6.—Nucleus in the resting stage with a single deeply staining nucleolus. Figs. 7–8.—Development of the spireme. Fig. 9.—Synizesis. Fig. 10.—Opening of the spireme. Fig. 11.—Open spireme stage. Fig. 12.—Metaphase of the heterotypic division; one mother-cell shows 20 chromosomes. Fig. 13.—Telophase of the heterotypic division, showing the formation of definite daughter nuclei and the persistence of the nucleolus of the parent nucleus. Figs. 14–15.—Homeotypic division, showing different arrangements of the spindles.

one side of the nuclear cavity. The nuclear karyolymph is finely granular, and there is no trace of any reticulum. Round the nucleolus is observed a small but distinct lighter zone which disappears gradually with the advancement of the division.

The heterotypic division commences with an increase in the density of the karyolymph, and soon after this the linin threads make their appearance in the form of a net-work at the periphery (Fig. 7). Numerous minute and faintly staining granules later become distinct on the linin, and it resolves into the usual reticulum (Fig. 8). It fills the whole nuclear cavity. After some time the thread begins to retract from the boundary, and contracts towards one side of the cavity into a knot. This contraction lasts for a fairly long time, but throughout this interval the outline of the thread can be made out. There is no sudden enlargement of the nucleus at this stage, it, therefore, does not support Lawson's (1911) view that synizesis results from an all of a sudden growth of the nuclear cavity. The growth is only slow and steady. The nucleolus is never enclosed in the synizetic knot but it lies near it on one side. The two have been seen connected with each other by a fine thread in many cases, which indicates that they are in a very close association with each other. In some cases small, spherical and deeply staining bodies, similar to "nucleolar bodies" recorded by Cleland (1922), Latter (1926), Sethi (1930), etc., have been observed lying at the circumference of the nucleolus. Occasionally these are found connected with the spireme (Fig. 9). During the contraction fragments of dark staining material are seen lying on the contracted thread, and its amount of chromatin increases. In some cases simultaneously with this and in other cases a bit later the staining capacity of the necleolus decreases (Figs. 5, 10 and 11). The decrease begins from the periphery towards the centre, and ultimately the whole body takes a very light stain. This behaviour suggests very strongly that during synizesis the nucleolus passes out its stainable material to the thread. Gregory, Wager, Cardiff, Sethi and many others (quoted in Sethi, 1930 and

Sharp, 1934) hold that the nucleolus serves as a store-house for the bulk of the chromatin material during the resting periods which intervene between successive mitoses. The same seems to be true for *Stellaria media*. The nucleus after the completion of the synizetic contraction enters the open spireme stage in which the thread seems to be continuous, and is comparatively more dense and thicker (Fig. 11). When just coming out of the contraction the thread shows concentration of chromatin at certain places to form numerous deeply staining bodies separated by lighter portions of the thread. Uptil this the thread seems to be univalent in nature and no split is perceptible at any place. No second contraction takes place. The thread is thrown into loops and this is followed by diakinesis stage. The exact changes which lead to the pairing of chromosomes could not be made out due to their rather large number and very minute size. Soon after the diakinesis the nuclear membrane and usually the nucleolus also disappear. Most of the further stages in meiosis were missing in the preparations examined. The number of bivalent chromosomes could be counted as twenty at the metaphase plate of the heterotypic division when cut across (Fig. 12). This confirms the haploid number as given by Rocén for the species. After the first telophase, the two groups of chromosomes on the two opposite poles of the spindle form two daughter nuclei with definite surrounding membranes (Fig. 13). No evanescent cell plate at the equator of the spindle could be made out. In one case two chromosomes were seen to lag behind on the spindle fibres while the daughter nuclei had been formed. In a few cases the primary nucleolus was seen to persist upto the late telophase (Fig. 13), which is rather curious. No wall is formed between the two daughter nuclei which again undergo the second meiotic division. The spindles of this division appear simultaneously and are mostly situated at right angles to each other (Fig. 14), but a parallel arrangement of them (Fig. 15) is also not rare. Soon after the four granddaughter nuclei have been formed at the poles of the two primary spindles, there appear secondary spindles of achromatic fibrils, and these connect the nuclei in all possible directions (Fig. 16). These secondary spindles persist for a considerable time. Cytokinesis takes place by cleavage of the cytoplasm (Fig. 17) in a manner as described by Farr (1916). No vacuoles similar to those reported by Castetter (1925) in *Melilotus* were seen.

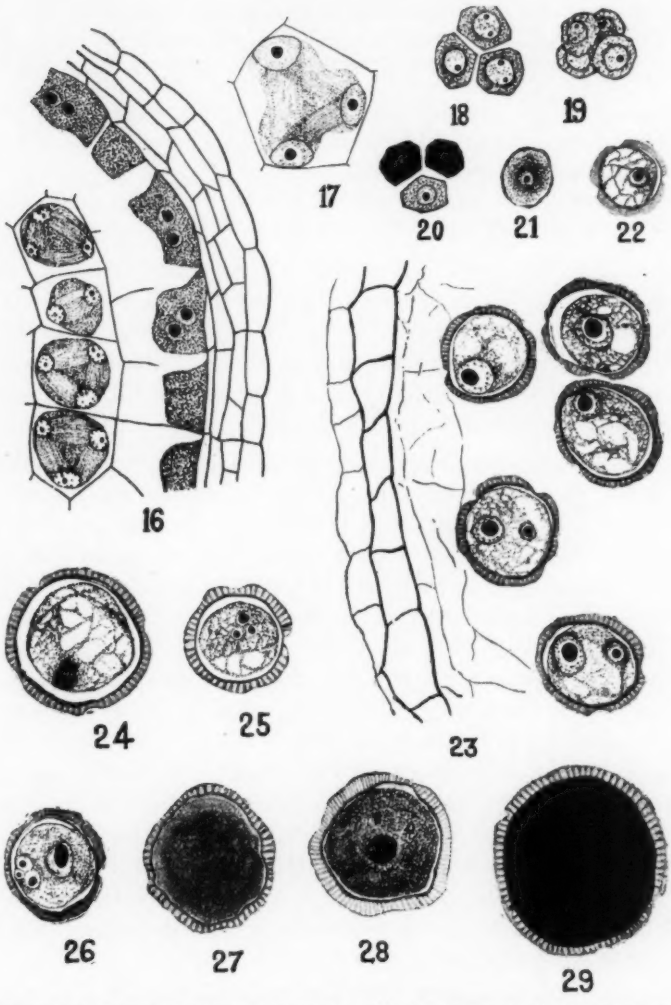
The pollen-grain.—The pollen-grains in the tetrad are arranged both tetrahedrally and bilaterally (Figs. 18–20). They get free from each other by the dissolution of the wall of the mother-cell. The degeneration of one, two, three or even four pollen-grains of a tetrad, while still inside the mother-cell wall, is quite common. The degenerated pollen-grains stain dark black.

The free microspores in the anther-halves round up and increase in size, and, as usual, a thick exine and a thin intine get differentiated, and the cytoplasm becomes vacuolated (Figs. 21–23). The nucleus divides into a large tube nucleus and a small generative nucleus (Figs. 23–24). No cell wall appears in between them, and the latter was never seen to organise itself into a cell. The further division of the generative nucleus of the pollen-grain inside the anther was seen to take place only in a few cases. It forms two similar male nuclei with definite nucleoli (Fig. 25). Perhaps the pollen-grains at the time of shedding are both binucleate and trinucleate.

The ripe pollen-grains vary much in size, measuring from 18μ to 30μ in diameter. Most of the large grains get degenerated (Figs. 27–29), their cytoplasm stains dark, and the nuclei undergo fragmentation. In some cases the nuclei were seen to divide in a mitotic manner even after the signs of degeneration had appeared (Figs. 27–28), and in one instance nearly twenty chromosomes could be counted very clearly. One normal pollen-grain was found to be four nucleate (Fig. 26). The extra nucleus was very small, and it occupied a position just near the pair of two male nuclei towards the periphery. In the Centrospermales a "prothallial cell" has been recently reported by Billings (1934) in *Atriplex hymenelytra*, but there is no scope for such an interpretation in the present case. According to Coulter and Chamberlain (1903), any cell (or nucleus) may be induced to divide under favourable conditions. In *Lilium*, *Asclepias*, etc., tube nucleus fragments, and forms additional nuclei. Schnarf (1929) thinks that all such instances of excessive nuclei in the male gametophyte, irregularities in meiosis and defective pollen development in angiosperms are mostly due to hybridisation.

The tapetum and all the wall layers of the anther except the outer epidermis and the endothecium begin to disintegrate after the formation of the pollen tetrads, and gradually disappear. The walls of the endothecial cells get thickened (Fig. 23), and in a mature anther these cells develop the characteristic fibrous bands.

Megasporogenesis.—The rudiments of the campylotropous ovules appear as small conical protuberances from the central placenta of the gynæceum in a basipetal succession as described by Gibbs. At first each of them consists of only a few homogeneous meristematic cells, but later the cells grow and multiply. The primary archesporium differentiates very early before the initials of the integuments are laid. It is hypodermal in origin and consists of four to six cells at the top of the nucellus. These are distinguishable, as usual by their large size, large nuclei and deeply staining



Shows various stages of microsporogenesis and development of the pollen-grain.

Fig. 16.—Part of l.s. of the anther, showing fully developed two-nucleate tapetum and the microspore mother-cells in telophase of the second meiotic division. × 900.

Fig. 17.—Shows cytokinesis by furrowing. × 1350.

Figs. 18–19.—Show the different arrangement of microspores formed from the same mother-cell. × 900.

Fig. 20.—Shows degeneration of two young pollen-grains in the tetrad. × 900.

Fig. 21.—Pollen-grain with a thin undifferentiated wall. × 900.

Fig. 22.—Pollen-grain, showing differentiation of the wall into intine and exine. × 900.

Fig. 23.—Part of l.s. of anther, showing thickening of the endothecial layer and complete differentiation of the wall of the pollen-grains, which are both 1- and 2-nucleate. × 900.

Fig. 24.—Pollen-grain with its nucleus in telophase of the first division. × 900.

Fig. 25.—Pollen-grain with three nuclei, the tube nucleus and the two male nuclei. × 900.

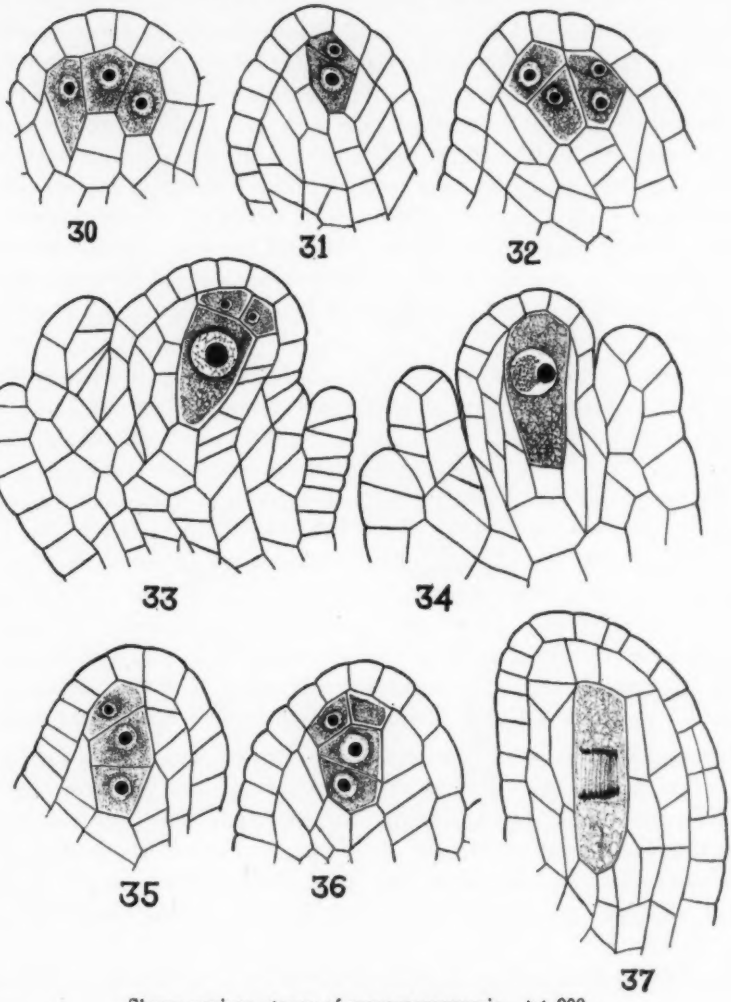
Fig. 26.—A 4-nucleate pollen-grain. × 900.

Figs. 27–29.—Degeneration of pollen-grains; two show mitotic division of nuclei even after the signs of degeneration have appeared. × 900.

cytoplasm. Fig. 30 shows the presence of three archesporial cells in a single longitudinal section of the ovule. The writer's observations are against the statement of Gibbs that there is only a single archesporial cell in the ovule of the species. *Silene, Melandrium, Agrostemma, Dianthus, Lychnis* and *Scleranthus* are other genera of the Caryophyllaceæ in which more than one archesporial cells have been noted (Schnarf, 1931).

All the primary archesporial cells of an ovule do not develop further in the same manner. Generally, only one or, less frequently, two located in the centre function; the rest lose their identity and become indistinguishable from the nucellar cells. The functional archesporial cell increases in size, and then divides transversely or obliquely to form the primary wall or parietal cell and the primary sporogenous cell (Figs. 31–33). The sporogenous cell, as usual, is itself a megaspore mother-cell. In very rare cases an archesporial cell enlarges and becomes a megaspore mother-cell without previously cutting off a parietal cell to the outside. This is apparent from Fig. 34 which shows a comparatively large cell with its nucleus in the synizesis stage of the first meiotic division and no wall cell on its outside. The non-formation of a wall cell has been described as a general condition by Gibbs in the Alsinoideæ and by Souèges (1926) in *Sagina procumbens*. Rocèn, on the other hand, has strongly contradicted these previous observations. Out of the numerous species studied by him, he could find only in case of *Gypsophila perfoliata* that sometimes the archesporial cell prepares for the heterotypic nuclear division without first separating off a wall cell.

The further development of the megaspore mother-cell is variable, and the results of the present investigation are at variance with those of Gibbs and Rocèn. A row of four or three megaspores, formed as a result of the usual two successive divisions of the mother-cell, was never seen. Only in a few cases the mother-cell had divided to form two superposed daughter cells with a transverse or an oblique wall in between (Figs. 35, 36). From a large number of slides examined nothing could be made out of the further behaviour of these two components of the dyad. Perhaps they do not



Shows various stages of megasporogenesis. × 900.

Fig. 30.—Apex of the nucellus, showing three cells of the primary archesporium.

Fig. 31.—One functional archesporial cell has divided to form a primary wall and a primary sporogenous cell (Megaspore mother-cell).

Fig. 32.—Two archesporial cells have divided to form a primary wall cell and a primary sporogenous cell.

Fig. 33.—Ovule showing the enlargement and elongation of the megaspore mother-cell.

Fig. 34.—The primary archesporial cell has developed into an embryo-sac cell without undergoing any division; nucleus in synizesis.

Figs. 35-36.—Dyad of two megaspores with an oblique or transverse wall in between.

Fig. 37.—The embryo-sac with its primary nucleus in telophase.

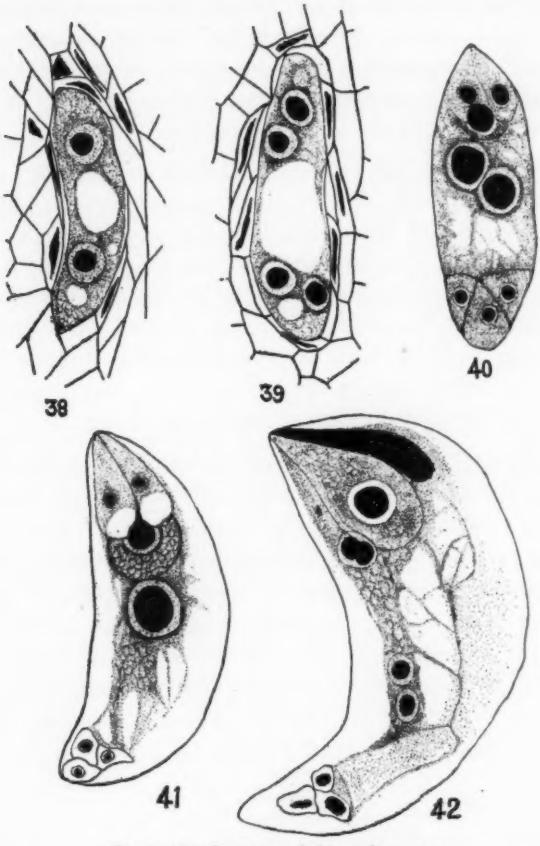
undergo any further division, but one of them enlarges to form directly the embryo-sac, the other merging into the nucellar cells in a manner similar to the non-functional archesporial cells. This probable suggestion does not preclude the possibility that the two megaspores may give rise to three or four megaspores which is held by Rocén as a constant condition for the species. In the majority of the ovules it is seen that the megaspore mother-cell does not divide at all, but itself functions as the initial of the embryo-sac. Figs. 34 and 37 show that it increases in size, and elongates rapidly as is usual with embryo-sacs in other plants. The primary nucleus divides into two. The division is not followed by wall formation and the result is a two-nucleate embryo-sac.

The above account shows that Gibbs and Rocén both are partly justified in their statements concerning megaspore formation in *Stellaria media*. Neither of them recorded any variation in the process or thought of its possibility. That is why contradictory statements have appeared, and this may be due to an examination of a small amount of material by each. On the other hand, externally *Stellaria media* is one of the most variable plants, and it may also be varying internally in this respect, in one variety one kind of megaspore formation may be more frequent. Rocén (1927) himself observed in *Mirabilis jalapa* and *Oxybaphus nyctagineus*, belonging to Nyctaginaceæ, rows of four, three and two megaspores and also the mother-cell functioning directly as a megaspore. It seems that this condition may be true for many more plants of the Centrospermales besides these examples.

The Embryo-sac.—One nucleus of the two-nucleate embryo-sac moves towards its micropylar end and the other towards the chalazal one. The embryo-sac continues to increase in size, especially lengthwise, and in the meanwhile vacuoles appear in the cytoplasm, and one in the middle portion of the embryo-sac becomes quite large (Fig. 38). The two nuclei at the two ends divide twice to produce the final eight nuclei of the adult embryo-sac (Figs. 38–40).

The fully-formed embryo-sac of *Stellaria media* is of the usual organisation, consisting of an egg-apparatus, three antipodals and two polar nuclei (Figs. 40, 41). Gibbs has said very little about these various components, and the diagrams given are too small to be fully illustrative, a brief account of them, therefore, seems desirable.

The egg-apparatus at the micropylar end consists of three elongated pear-shaped cells, the egg being somewhat larger than the two synergids. In a frontal view the synergids lie side by side almost touching by their lateral walls, and they cover a major portion of the egg, only its rounded distal end



Shows development of the embryo-sac.

Fig. 38.—A two-nucleate embryo-sac; some of the cells on the periphery of the embryo-sac have been disintegrated by its enlargement. × 900.

Fig. 39.—A 4-nucleate embryo-sac. × 900.

Fig. 40.—A young fully-formed embryo-sac (Re-constructed). × 900.

Fig. 41.—Embryo-sac at a later stage; the antipodals are in a process of degeneration (Re-constructed). × 450.

Fig. 42.—Embryo-sac after fertilisation, showing the remains of a pollen tube on one side of the undivided oospore, a few endosperm nuclei and the last remains of antipodals; the synergids have disintegrated altogether (Re-constructed). × 450.

being visible. During early stages of development the three cells of the egg-apparatus are seen completely full of cytoplasm, but, as usual, with continued growth they become vacuolated. In each synergid a large vacuole appears in the basal region of the cell, and the nucleus is embedded in

cytoplasm just above this vacuole. The synergids are devoid of every kind of filiform apparatus or hooks. In case of the egg the vacuole appears in the apical region, and its nucleus lies in the broad basal part of the cell surrounded by an extremely dense cytoplasm. The mature egg very much exceeds a synergid in the size of its cell and the nucleus.

The three antipodal cells at the chalazal end of the embryo-sac are more or less triangular in shape. Their arrangement is variable ; generally, they lie close to one another at the same height, but in some cases one lies just at the bottom of the embryo-sac and the other two lie above it in close proximity. Each antipodal is at first surrounded externally by a membrane like the cells of the egg-apparatus, but later it becomes covered by a definite cell wall. The antipodals of *Stellaria media* are ephemeral in nature like those of other caryophyllaceæ. They begin to degenerate early, and disappear altogether about the time of the first division of the fertilised egg.

The two polar nuclei, as described by Gibbs, fuse at the micropylar end to form a very large secondary nucleus which comes to lie very close to the egg.

Gibbs thinks that after fertilisation the first division of the oospore always precedes the first division of the endosperm nucleus, but the writer has found the reverse to be a constant condition in all the preparations examined for the purpose (Fig. 42). At the time of the first segmentation of the egg there are always found four to eight free endosperm nuclei in the embryo-sac. The division of the endosperm nuclei is mitotic, but sometimes figures similar to those of the amitotic one are also seen.

4. Summary.

1. The primary archesporium in each sporangium of the anther of *Stellaria media* consists of several cells.

2. Microsporogenesis is almost typical. The mother-cells do not round off and separate from one another prior to division. During synizesis staining material of the nucleolus appears to pass out to the spireme which gains in its chromatin content. Cytokinesis takes place by furrowing.

3. The tapetum is formed from parietal tissue alone. At about the time of diakinesis the nuclei of the tapetal cells divide mitotically, and the tapetal cells become binucleate.

4. The pollen output of each anther appears to be about 320. The pollen grains are perhaps shed in both 2- and 3-nucleate condition. The generative nucleus has not been seen to organise a cell.

5. The primary archesporium in the ovule consists of 4–6 cells, one of which functions ultimately.

6. Usually the functional archesporial cell divides to form a primary wall cell on the outside and a primary sporogenous cell (megaspore mother-cell) on the inside, but in rare cases no wall cell is cut off.

7. The megasporogenesis is variable. In no case a linear row of 4 or 3 megaspores was seen, but in a few cases a dyad formed from a mother-cell was seen. In majority of cases a mother-cell becomes itself the embryo-sac cell.

8. The fully-formed embryo-sac is an 8-nucleate structure of the usual organisation.

9. The primary endosperm nucleus always divides before the fertilised egg.

The writer is highly indebted to the late Prof. S. R. Kashyap for the suggestion of the problem and guidance throughout its progress. Sincere thanks are due to Dr. S. L. Ghose for his keen interest during the course of investigation and in revising the manuscript and giving several critical suggestions. The writer is also indebted to Prof. A. C. Joshi of Benares Hindu University and Mr. P. N. Mehra for rendering valuable help.

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THE MUSSALMANS OF GOA.

(Anthropological Study.)

By Lt.-Col. Prof. Alberto Carlos Germano da Silva Correia.
(Nova-Goa)

Received December 3, 1935.

Before the Muhammadan invasion of the Deccan, in the latter end of the thirteenth century Islam had not made much progress there.

After this period the Deccan was overrun by Muhammadans. During the Bahmani Empire of the Deccan and Konkan (1347–1526) many foreign troops such as Arabs, Tartars, Afghans and Persians were introduced.

Some of these settled in the country and from them have sprung the Deccani Mussalmans and consequently the Goanese Muhammadans, either by a continued residence or by union with the women of the country.

The Goanese Mussalmans belong to the great group of the Deccani Mussalmans.

The anthropometric study on the Goanese Muhammadans concerns 100 individuals, of the masculine sex, inhabiting the territory of the Indo-Portuguese district of Goa.

Those subjects whose somatic measurements I have taken were all soldiers and motor-car drivers.

I have observed the following anthropometric data :

Crucial Index.—The global average is of 106,25 nearer to those which characterize the white race than to those of the negro race.

Crural Index.—The average is 50,46, indicative of a mesosquelic type.

Horizontal Cephalic Index.—The average is of 79,51 indicative of a mesocephalic type.

Schmidt's Module.—This average is of 157,74, indicative of medium-sized heads.

Total Facial Index.—The average is of 90,17, designative of the mesoprosopy.

Superior Facial Index.—The average is of 55,22, indicative of the mœsenic type.

Nasal Index.—With an average of 70,95, indicative of the leptorrhinian type.

Ponderal Index.—With an average of 23,3, indicative of sub-normal ponderal type.

Size or Height.—It is of 1^{m}, 656^{mm} indicative of a group over than the medium type.

Dynamometry.—The bimanual dynamometric average to the pressure is of 36 Kilograms.

Anthropo-typology.—As for the disposition predominate the mixed and sangüineous physiological types, and concerning the noso-morphologic types preponderate among the Goanese Mussalmans the *mixed type* in the first place, and afterwards the *muscular type* respectively with the percentages of 48% and 30%, the remaining individuals belonging to the nervous and lymphatic types.

Skin Colour.—There prevails the XV, XVI, XVIII, XX and XXII of Von Lueschan's scale, that is indicative of whitish colour.

General Morphological Aspect.—Men of size over than medium type, regularly strong, dolichocephalic, leptorrhinian, well proportionate and little communicative.

Hirszfeld's Index.—Concerning the blood groups among them I have calculated the *Bio-chimic index of Hirszfeld* and I have found the average of 0,88, higher than that I have calculated for the Goanese Mahrattas.

Conclusion.—It seems to me that the Goanese Mussalmans are a mixed ethnic group, issued of the race crossing between the Arabs mostly, and the Hindus inhabiting the Malabar, Deccan and Konkan. The pure descents of Arabs firstly, and of the Afghans secondly, are very rare, and, practically it is quite impossible to find them in this country.

A REVIEW OF THE GENUS *PARAMONOSTOMUM*, LÜHE; WITH DESCRIPTIONS OF TWO NEW SPECIES AND REMARKS ON THE GENERA OF THE SUB-FAMILY NOTOCOTYLINAE.

By Makund Behari Lal, M.Sc.
(From the Department of Zoology, Lucknow University.)

Received December 14, 1935.
[Communicated by Dr. G. S. Thapar, M.Sc., Ph.D. (London).]

Although good deal of work has been done on the trematode parasites of birds, there is, as will appear from the following pages and papers to be published subsequently, a certain amount of confusion in the morphology and nomenclature of the trematodes of this group of Vertebrates. I, therefore, undertook at the suggestion of Dr. G. S. Thapar, the study of some forms that were available from birds at and about Lucknow.

In my communications on the genus *Notocotylus*, I have laid emphasis on the importance of the position of genital pore in the generic diagnosis and as a result, the genus *Notocotylus* has been revised, with the consequent creation of two new genera—*Hindia* and *Naviformia*. The present communication reveals further the importance of this character in the diagnosis of genera in the sub-family Notocotylinæ and lends support to the view already expressed. Thus, while two new species of the genus *Paramonostomum* are being described, advantage is taken to review the hitherto known species of the genus *Paramonostomum* on the basis of this character. It is hoped that this may remove the existing confusion with regard to the forms described under the sub-family Notocotylinæ.

I may here express my indebtedness to Dr. G. S. Thapar for his guidance and valuable suggestions during the course of this work.

Genus *Paramonostomum*.

The genus *Paramonostomum* was erected by Lühe (1909) to include *Monostomum alveatum* (Mehlis) Creplin and is characterised mainly by the complete absence of the ventral glands that are present in other members of the sub-family Notocotylinæ. Barker (1916) questioned the validity of this genus created on the absence of the ventral glands, but it is strange to find that he accepts the genus *Catatropis* based mainly on the non-protrusibility of these glands. There appears to me a greater reason to accept a genus

formed on the absence of an organ than to accept one founded on its non-protrusibility. I, therefore, agree with Harrah and other workers in recognizing the genus *Paramonostomum* as valid. The following two new species are described under this genus :—

Paramonostomum querquedulum n.sp.

This trematode was obtained from the intestinal cæca of the Garganey, *Querquedula circia*. It measures 3·398 mm. in length and has a maximum width of 1·11 mm. in the middle region of the body behind the cirrus sac.

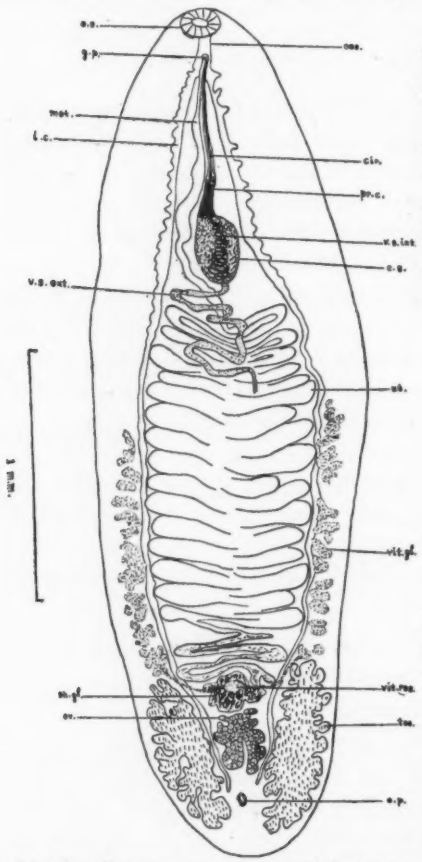

FIG. 1. *Paramonostomum querquedulum*, n.sp., showing the disposition of the internal organs.

The oral sucker is sub-ventral and measures ·17 mm. by ·12 mm. and is followed by an elongated œsophagus, ·165 mm. long. At its posterior end the œsophagus divides into two intestinal cæca which run upto the posterior end of the body. The intestinal cæca at their proximal end show slight irregular projections along their external border.

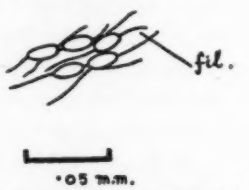

FIG. 2. *Paramonostomum querquedulum*, n. sp., eggs with filaments.

The excretory system is as usual, with an excretory pore at the posterior end of the body behind the ovary.

The testes are two in number, and extracæcal in position. Their outer margin is deeply lobed. The left testis is slightly larger than the right and measures ·74 mm. by ·235 mm. The right testis measures ·66 mm. by ·24 mm. The two vasa deferentia meet in the middle to form the vesicula seminalis consisting of a proximal portion (vesicula seminalis externa) and a distal portion enclosed within the cirrus sac (vesicula seminalis

interna). The cirrus sac is pear-shaped with long neck and measures ·93 mm. in length. It contains a long but feeble cirrus and a few prostatic gland-cells near the base of its neck.

The ovary which is more or less bifid posteriorly measures ·265 mm. by ·22 mm. The öotype is situated in front of the ovary and receives ducts from the vitelline glands. The shell-gland cells are present and surround the öotype and the vitelline reservoir. The vitelline glands consist of separate follicles mostly extracæcal, and occupy about the middle region of the animal. The two horizontal vitelline ducts of the two sides unite in the region of the öotype to give rise to the vitelline reservoir. The uterus arises from the left side of the öotype and runs forward forming transverse loops ending in the metraterm at its distal end. The metraterm runs parallel to the cirrus sac and opens with it at the genital pore which is situated midway between the oral sucker and the intestinal bifurcation. The uterus is full of thin-shelled eggs that are provided with a filament at their either end. They measure ·02 mm. by ·009 mm. without filaments.

This species is characterised thus :—

1. Monostomes without ventral glands.
2. Genital pore situated midway between the oral sucker and the intestinal fork.
3. The ovary bifid posteriorly and the uterus with transverse loops in the intercæcal region.
4. Vitellaria extend slightly beyond the middle of the body from the posterior end.
5. The intestinal cæca show slight lateral projections on their outer margin.

This species differs from the type species *Paramonostomum alveatum* and from the species *Paramonostomum pseudalveatum* in the proportion of length and breadth of the body, in the general topography of the organs, and specially in the deeply-lobed condition of the testes, the extent of vitellaria, and greater number of uterine loops. Hence it is designated as a new species.

Paramonostomum casarcum n.sp.

This trematode was obtained from the intestinal cæca of the Brahminy duck, *Casarca rutila* and appeared pink in fresh condition. It measures 3·8 mm. by ·95 mm. The body is quite flat with the anterior end narrower than the posterior.

The oral sucker, situated ventrally at the anterior end, measures ·14 mm. by ·1 mm. and is followed by œsophagus ·14 mm. long. The œsophagus divides into two intestinal cæca which run to the posterior end of the body.

The excretory pore lies midway between the ovary and the posterior end. It is a small slit-like aperture in the middle in between the last lobe of the testes.

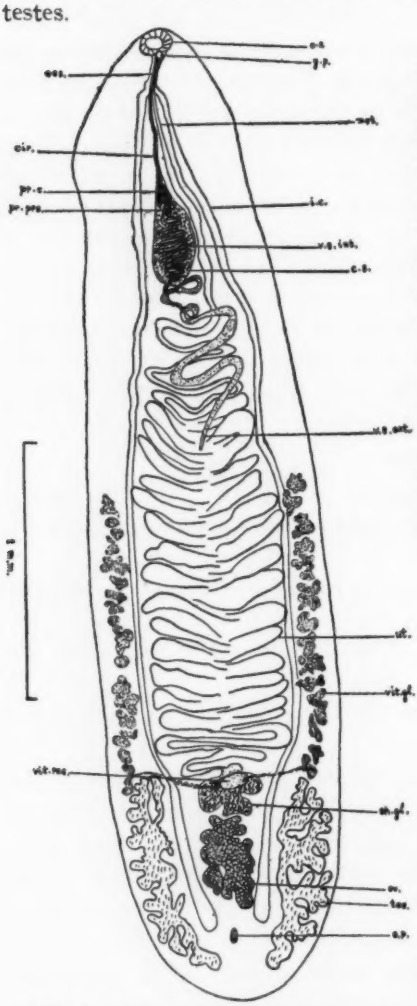

FIG. 3. *Paramonostomum casarcum*, n.sp., showing the disposition of the internal organs.

The testes are deeply lobed on their external margins. They are of different sizes—the right testis, measuring ·8 mm. by ·15 mm., is slightly longer than the left which measures ·76 mm. by ·175 mm. The vasa deferentia arising from the testes join together anteriorly to form the vesicula seminalis. The vesicula seminalis interna occupies the base of the cirrus sac which is tubular, wide at the base, and measures ·91 mm. in length. The genital aperture opens at the posterior border of the oral sucker. The cirrus is long and muscular. The pars prostatica and the prostatic cells are present.

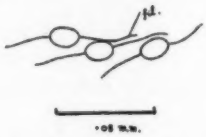

FIG. 4. *Paramonostomum casarcum*, n.sp., eggs with filaments.

The ovary is deeply lobed and measures ·38 mm. by ·22 mm. The öotype is situated in front of it. The shell-glands consist of small gland-cells and occupy a large area in the region of the öotype. The vitellaria are in the form of separate follicles placed in the usual position and extend from the anterior tip of the testes forward beyond the middle of the body. Their horizontal ducts meet in the region of the öotype to form a vitelline reservoir, that opens into the öotype. The uterus seems to arise from the right side of the anterior boundary of the öotype. It runs forward forming loops and finally forms the metraterm which runs parallel to the cirrus sac and opens

along with the latter at the genital aperture. The eggs measure ·015 mm. by ·01 mm. without filament. Each egg is thin-shelled and provided with a filament at each pole.

To sum up, the characters of the species are :—

1. Monostomes without ventral glands.
2. Genital pore at the posterior border of the oral sucker.
3. Uterus with transverse loops in the intercæcal region in front of the öotype.
4. Vitelline glands extend beyond the middle of the body from the posterior end.

This species differs from *Paramonostomum alveatum* and *Paramonostomum pseudalveatum* in the deeply lobed condition of the testes, the extent of the vitellaria, and the greater number of the uterine loops ; from the allied species *Paramonostomum querquedulum* it differs in the forward position of the genital pore, in the proportionate width of the animal, and the size of the eggs. It is, therefore, described as a new species.

Discussion.

Besides the two new species of the genus *Paramonostomum* described above, eight other species have so far been described under this genus. They are :—

Paramonostomum alveatum (type species).
Paramonostomum pseudalveatum.
Paramonostomum ionorne.
Paramonostomum echinum.
Paramonostomum elongatum.
Paramonostomum parvum.
Paramonostomum microstomum.
Paramonostomum bucephale.

They can be arranged into two distinct groups as regards the position of their genital pore :

1. Genital pore in front of the intestinal fork.
   - *Paramonostomum alveatum.*
   - *Paramonostomum pseudalveatum.*

   (To this list are being added two new species described above, *viz.*, *Paramonostomum querquedulum* and *Paramonostomum casarcum.*)

2. Genital pore behind the intestinal fork.
   - *Paramonostomum ionorne.*
   - *Paramonostomum echinum.*
   - *Paramonostomum elongatum.*
   - *Paramonostomum parvum.*
   - *Paramonostomum microstomum.*
   - *Paramonostomum bucephale.*

The type species of the genus, *Paramonostomum alveatum*, has its genital pore in front of the intestinal fork. Evidently, therefore, only those species of the genus should be included under it that agree with the type species in general characters including the position of the genital pore. Other species have, evidently, been misplaced and their authors appear to have taken a great latitude in including them under this genus. The writer, therefore, suggests to split the genus *Paramonostomum* in the same way as he has done in *Notocotylus*. The so-called genus *Paramonostomum* is divided into two genera as follows :—

Genus *Paramonostomum* Sens. Str.

Type species—*Paramonostomum alveatum.*

Characters of the Genus.—Monostomes *without ventral glands* and a pharynx; intestinal cæca run almost upto the posterior end of the animal ; testes may or may not be lobed, extra-cæcal in the posterior part of the body ; part of the vesicula seminalis enclosed within the cirrus sac ; ovary in between the testes ; shell-glands pre-ovarial ; uterine loops mostly confined to the intercæcal region behind the cirrus sac ; receptaculum seminis absent ; *genital pore always opens in front of the intestinal fork and behind the oral sucker ; vitelline glands never end behind the midbody from the posterior end ;* eggs with filaments at either end.

Other species :—

Paramonostomum pseudalveatum.
Paramonostomum querquedulum.
Paramonostomum casarcum.

Key for the Identification of the Species of the Genus, Paramonostomum *Sens. Str.*

1. Uterine loops few, about 6-8 2
   Uterine loops always more than 15 .. 3
2. Vitellaria upto equator of body .. .. *P. alveatum.*
   Vitellaria upto $\frac{2}{3}$ of the body .. .. *P. pseudalveatum.*
3. Genital pore at the base of oral sucker, intestinal cæca show slight projections at their anterior end .. .. *P. querquedulum.*
   Genital pore much behind oral sucker, intestinal cæca show no such projections .. .. *P. casarcum.*

Genus *Neoparamonostomum* is created for those forms which have the genital pore behind the intestinal fork.

Type species—*Neoparamonostomum ionorne*
(Syn. *Paramonostomum ionorne* Travassos).

Characters of the Genus.—Monostomes *without ventral glands* and pharynx; intestinal cæca run almost upto the posterior end of the animal ; testes extra-cæcal and posterior and generally lobed ; part of the vesicula seminalis enclosed within the cirrus sac ; ovary in between the testes ; shell-glands pre-ovarial ; uterine loops mostly confined behind the cirrus sac ; receptaculum seminis absent ; *receptaculum seminis uterinum may be present ; genital pore always opens behind intestinal fork ; vitelline glands end behind midbody from the posterior end* ; eggs with filaments at either end.

Other species :—

Neoparamonostomum echinum (Syn. *Paramonostomum echinum*).
Neoparamonostomum parvum (Syn. *Paramonostomum parvum*).
Neoparamonostomum elongatum (Syn. *Paramonostomum elongatum*).
Neoparamonostomum microstomum (Syn. *Paramonostomum microstomum*).
Neoparamonostomum bucephale (Syn. *Paramonostomum bucephale*).

Key for the Identification of the Species of the Genus Neoparamonostomum.

1.	Length and breadth almost equal, size conspicuously small, about ½ mm. ..	*N. parvum.*
	Length several times the breadth	2
2.	Intestinal cæca present short internal and external diverticula	*N. echinum.*
	No such diverticula present	3
3.	Vitellaria upto level of base of cirrus sac ..	*N. ionorne.*
	Vitellaria never reach level of base of cirrus sac	4
4.	Vagina as long as cirrus sac	*N. microstomum.*
	Vagina shorter than cirrus sac	5
5.	Cirrus covered with tubercles	*N. bucephale.*
	Cirrus smooth, not covered with tubercles ..	*N. elongatum.*

Characters of other genera of the sub-family Notocotylinæ.

Genus *Notocotylus* Sens. Str.*—*Monostomes with two to five rows of protrusible ventral glands* ; pharynx absent ; testes extra-cæcal and posterior ; ovary in between testes ; shell-glands pre-ovarial ; uterine loops confined to the inter-cæcal region behind the cirrus sac ; receptaculum seminis absent ; part of the vesicula seminalis enclosed within the cirrus sac ; *cirrus sac never extending beyond half of body length ; vagina about ⅓ to as long as cirrus sac ; genital pore situated behind the intestinal bifurcation ; vitelline glands extend upto the middle of the body* ; eggs ·018–·022 mm. long with filaments at either end. Type species—*Notocotylus attenuatus.*

* For the identification keys of this genus, refer to Lal, M. B., *Proc. Ind. Acad. Sci.*, 1935, Vol. 2, No. 5.

Genus *Hindia*.*—Monostomes *with three rows of protrusible ventral glands;* pharynx absent ; testes extra-cæcal and posterior ; ovary in between testes ; shell-glands pre-ovarial ; uterine loops lie in the inter-cæcal region behind the cirrus sac ; receptaculum seminis absent ; part of the vesicula seminalis enclosed within the cirrus sac ; *cirrus sac extending about* $\frac{1}{4}$ *to* $\frac{1}{2}$ *body length ; vagina never more than* $\frac{3}{4}$ *of the cirrus sac ; genital pore at the intestinal fork ; vitelline glands extend* $\frac{1}{3}$ *to* $\frac{1}{2}$ *of the body length from the posterior end* ; eggs ·014–·0209 mm. long, with filaments at either end.

Type species—*Hindia gibbus.*

Genus *Naviformia.*—Monostomes *with three rows of protrusible ventral glands ;* pharynx absent ; testes extra-cæcal and posterior ; ovary in between the testes ; shell-glands pre-ovarial ; uterine coils lie in the inter-cæcal region behind the cirrus sac ; receptaculum seminis absent ; part of the vesicula seminalis is enclosed within the cirrus sac ; *cirrus sac extending about* $\frac{1}{3}$ *of the body length ; vagina* $\frac{4}{5}$ *of the cirrus sac ; genital pore in front of the intestinal fork ; vitelline glands behind midbody* ; eggs ·0178–·0208 mm. long, with filaments at either end.

Type species—*Naviformia naviformes.*

Only one species described under this genus.

Genus *Catatropis.—Monostomes with three rows of weakly developed non-protrusible ventral glands ; the middle row of ventral glands may be placed on a median keel or a ridge* ; pharynx absent ; testes extra-cæcal and posterior ; ovary inter-testicular ; shell-glands pre-ovarial ; vesicula seminalis is divisible into an external and an internal portion ; receptaculum seminis absent ; *vagina is very much developed, about as long as cirrus sac ; genital pore situated behind the intestinal fork* ; eggs with filaments at either pole.

Type species—*Catatropis verrucosa.*

Key for the Identification of the Genera of the Sub-Family Notocotylinæ :

1.	Ventral glands present ..	..	2
	Ventral glands absent ..	..	3
2.	Ventral glands protrusible ..	..	4
	Ventral glands non-protrusible ..	..	*Catatropis.*
3.	Genital pore between oral sucker and intestinal fork ..	..	*Paramonostomum.*
	Genital pore behind intestinal fork	..	*Neoparamonostomum* n.g.

* For the identification keys of this genus, refer to Lal, M. B., *Proc. Ind. Acad. Sci.*, 1935, Vol. 2, No. 5.

4. Genital pore behind intestinal fork .. *Notocotylus.*
Genital pore at the intestinal fork .. *Hindia.*
Genital pore in front of intestinal fork .. *Naviformia.*

LIST OF ABBREVIATIONS USED IN THE FIGURES.

cir.	.. Cirrus.	*pr.c.*	.. Prostatic cells.
c.s.	.. Cirrus sac.	*pr. prs.*	.. Pars prostatica.
e.p.	.. Excretory pore.	*sh. gl.*	.. Shell-glands.
fil.	.. Filament of egg.	*tes.*	.. Testis.
g.p.	.. Genital pore.	*ut.*	.. Uterus.
i.c.	.. Intestinal cæca.	*v.s. ext.*	.. Vesicula seminalis externa.
met.	.. Metraterm.	*v.s. int.*	.. Vesicula seminalis interna.
o.s.	.. Oral sucker.	*vit. gl.*	.. Vitelline glands.
oes.	.. Oesophagus.	*vit. res.*	.. Vitelline reservoir.
ov.	.. Ovary.		

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ESTIMATION OF NITROGEN BY FUMELESS DIGESTION. PART II.

Products of Oxidative Digestion of Organic Nitrogen and the Procedure for their Inclusion in the Estimate of Total Nitrogen.

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It was shown in the previous communication (Narayanayya and Subrahmanyan, 1933[3]) that the digestion of organic nitrogen can be greatly accelerated by addition of oxidising agents. Attention was also drawn to the fact that the digestion proceeds to completion in presence of water, so that emission of fumes which is the most objectionable feature of the Kjeldahl method (or any of its modifications) is entirely eliminated. Based on these and other observations, a simple and rapid method for the estimation of nitrogen has been developed and shown to be applicable to soils and biological materials in general.

Although the above findings are of considerable practical interest, further information is, nevertheless, needed regarding the nature of the products formed during oxidative digestion and the manner in which they are included in the estimate of total nitrogen. Some of the conditions relating to the digestion also require elucidation and the details of procedure to be further simplified so as to facilitate easy adoption in routine practice. These and related problems have been studied and will be discussed in the present communication.

Experimental.

It was suggested in some earlier communications (Narayanayya and Subrahmanyan, 1935[1,2]) that the material to be digested may be heated together with dichromate, water and sulphuric acid. When applied in practice, however, such a procedure was not found to be satisfactory. Thus, when mixtures of soil, dichromate and acid (2 : 1) were heated gently to boiling, the digests invariably yielded lower results than those obtained by the Kjeldahl "wet" method (Sreenivasan, 1932). The error was not constant but varied in the hands of different workers. The following results (Table I) obtained

with one specimen of soil will suffice for an illustration. The soil (a local specimen ; 10 g.) was treated with potassium dichromate (5 g.), mercuric oxide (2 g.), water (20 c.c.) and sulphuric acid (40 c.c.) in succession and the mixture boiled for 30 minutes. The digest was treated with zinc (10 g.) and boiled for 15 minutes. It was then cooled and distilled with excess of alkali in the usual way.

TABLE I.

Observations by	Forms of zinc used for reduction	Alkali equivalent of nitrogen (as c.c. of 0·0352 N alkali)		Total Nitrogen in parts per million (Average estimate)
		Correction for zinc	Total nitrogen in digest	
A	Dust	6·3	16·1, 16·3	488
B	Do. (same sample)	6·2	16·3, 16·4	498
C	Do.	6·1	15·8, 16·0	483
D	Do.	5·5	15·6, 15·35	493
A	Turnings	0·6	10·1, 10·0	466
B	Do. (same sample)	1·0	10·7, 10·65	477
C	Do.	0·3	10·45, 10·35	498

A, B, C,.....were different workers. The value expected according to the Kjeldahl wet' method was 518 p.p.m.

The digestion was apparently complete in all the cases, as may be seen from the separation of white sand. The distillation was also quite smooth.

It was observed, however, that zinc was not digested to the same extent in all the cases. This was largely due to the presence of mercury, which formed a coat of hydride around unattacked zinc and thus retarded its reaction with acid. It is probable that this may have rendered the blank for zinc so variable. Even after allowing the minimum blank for zinc, the estimate of total nitrogen in the soil was still too low as compared with the expected value.

The discordant nature of the results, though coming within a narrow range, still suggested that certain conditions relating to the digestion were not adequately controlled. Further work was therefore carried out with

a view to throwing light on the nature of factors determining the efficiency of digestion.

Effect of addition of mineral catalysts.—Narayanayya and Subrahmanyan (*loc. cit.*) found that the acid digestion of cyanamide was catalysed by the presence of certain mineral salts. With a view to determining whether the estimate of nitrogen can be improved by such treatments, small quantities of these catalysts were added to mixtures of soil (10 g.) and acid (60 c.c., 2 : 1). In one set of trials, the mixtures were heated for 15 mins. under reflex and then cooled before addition of dichromate. In another set, the dichromate was added together with the catalyst and the mixtures raised to boil. After boiling for 30 mins., the digests were reduced with zinc (10 g.) and distilled with excess of alkali. The results (after deducting the average blank for zinc as expressed in parts per million) were as follows:—*Catalyst added before dichromate.* (*a*) *Potassium sulphide* (10 g.) *added together with alkali.* $MnSO_4$ (2 g.), 735 p.p.m.; $CuSO_4$ $5H_2O$ (2 g.), 720; $CuSO_4$ $5H_2O$ (2 g.) + $HgSO_4$ (3 g.), 695. (*b*) *Potassium sulphide not added with alkali.* $CuSO_4$ $5H_2O$, 710; $MnSO_4$, 700; SeO_2, 715; BaO_2, 705. *Catalyst added together with dichromate.* $CuSO_4$ $5H_2O$ (2 g.) + $HgSO_4$ (3 g.), 666; $CuSO_4$ $5H_2O$, 656. Value expected (Kjeldahl 'wet' digestion), 764. It may be seen from the above that none of the treatments yielded the correct estimate for total nitrogen. The low estimates of total nitrogen should therefore be traced to other causes.

Nitrogen content of zinc.—It was found that even the purest specimens of zinc contained some nitrogen. The blanks were also variable. It was further noted that if the zinc was boiled with acid and the digest distilled with excess of alkali, the blank for nitrogen was slightly higher than when the metal was boiled directly with alkali. These observations would suggest that the blank for zinc, especially when used in such large quantities as 10 grams, was unreliable.

Attempts to remove the nitrogen associated with zinc were unsuccessful. A leading European manufacturing firm was consulted in the matter, but they were also unable to supply nitrogen-free zinc. It was considered necessary, therefore, to either eliminate the use of zinc altogether or, at any rate, apply it in such small quantities that the error due to the nitrogen present in the metal would be negligible.

Reduced iron.—It has already been shown (Narayanayya and Subrahmanyan, *loc. cit.*) that reduced iron can be used in place of zinc, though the formation of the heavy precipitate in alkaline medium renders the distillation rather difficult. In spite of this disadvantage, a few trials were carried out, using reduced iron either as such or after boiling with alkali (50 per cent.) to

remove as much of the associated nitrogen as possible. Two different specimens of soils (in 10 g. lots) were taken and, after being treated in the manner outlined previously, the digests were diluted with water, boiled with reduced ion (10 g.) and then distilled with alkali in the usual way. The results have been given in Table II.

TABLE II.

Material used for reduction	Alkali equivalent of nitrogen (As c. c. of 0·0352 N alkali)		Total nitrogen in soil (in parts per million)	
	Correction for N in iron	Total N in the digest	As found	As expected
Reduced iron (as such)	0·85	11·95	547	569
Do.	0·75	11·75	542	,,
Do.	0·60	11·50	537	,,
Do. (after boiling with alkali)	0·40	15·55	747	764
Do.	0·40	15·15	727	,,
Do.	0·40	15·75	757	,,

It may be seen from the above that reduced iron also contained nitrogen, though not in such large quantities as zinc dust. Boiling with alkali proved highly beneficial because, in addition to removing a part of the nitrogen and thus yielding a consistent blank, it also dissolved out the arsenic with which the iron was associated. If arsenic was not removed in this manner, the poisonous gas, arsine, used to be formed in considerable quantities and was given off freely both during boiling with acid and subsequent distillation with alkali.

As observed in the previous experiment, the final estimates of nitrogen were too low even after correction for the nitrogen content of the iron used for reduction. The deviations from the expected values depended largely on the mode of heating, though it was not possible to define as to how it was affected in each case.

The nature and extent of reduction needed to release nitrogen from combination.—The residue after oxidative digestion contains (*a*) unused chromic acid, (*b*) compounds containing nitrogen in association with chromium

mercury and other mineral constituents in the medium, and (*c*) nitric acid, part of which may have been derived from the nitrites and nitrates originally present in the medium and part formed through the oxidation of organic nitrogen. Of these three, the first requires no explanation though it may be pointed out that, on prolonged heating with acid, chromic acid will undergo steady decomposition, yielding oxygen, and thus getting reduced to the chromic (Cr····) condition. This reaction takes, however, such a long time, especially in presence of water, that it may not be convenient, in practice, to reduce the unused chromic acid in that manner. Resistant compounds of mercury containing nitrogen are well known. The associated nitrogen can be released by either precipitation of mercury as the sulphide or by treating the digest with a reducing agent as suggested by Narayanayya and Subrahmanyan (*loc. cit.*). The latter procedure has also been applied for release of nitrogen occurring in association with chromium though it is not clear whether nascent hydrogen—as formed through treatment with zinc or reduced iron—is required for the purpose. Minute quantities of nitric acid are formed during oxidative digestion (Harihara Iyer, Rajagopalan and Subrahmanyan, 1935). The quantities actually formed—at any rate in the case of most soils—are so small that even if the digest is boiled in an open flask, the estimate of total nitrogen will not be appreciably affected. It is probable, however, that as recently reported by Acharya (1935), larger quantities of nitric acid may be formed from other biological materials.

Of the three above-mentioned forms, the unused chromic acid which reacts with the major part of zinc or reduced iron can be reduced equally efficiently by oxalic acid, stannous salts, sulphites and such other reagents. It is difficult to state whether the nitrogen associated with mercury or chromium can also be released by such reagents. Nitrate nitrogen cannot be reduced to ammonia by such compounds. It thus appeared probable that one of the above-mentioned reagents—which may be expected to be reasonably free from nitrogen—can be used in conjunction with zinc or reduced iron in acid medium or Devarda's alloy or aluminium in alkaline medium. Some preliminary trials were accordingly carried out to determine the extent to which the different chemicals contained combined nitrogen as an impurity. In each case, a mixture containing dichromate (5 g.), mercuric oxide (2 g.), water (20 c.c.) and sulphuric acid (40 c.c.) was refluxed under water-cooled condenser for 30 mins. after which the unused chromic acid was reduced with excess of one of the reagents. The products were then cooled, treated with excess of alkali and distilled in the usual way. The average alkali (0·0352 N) equivalent of the ammonia distilling over in each case was as follows :— oxalic acid (pure), nil ; sodium sulphite (A.R.), nil ; potassium sulphide,

nil; stannous chloride (1st sample), nil; stannous chloride (2nd sample, 15 g.), 1·90 c.c.; stannous sulphate (15 g.), 2·30 c.c.; ferrous sulphate (15 g.), 0·65 c.c.; and hydrogen peroxide (10 c.c., 30 per cent.), 1·35 c.c.

Of these, oxalic acid acted fairly rapidly on heating the diluted acid digest. Sodium sulphite acted readily in the cold and there was no smell of sulphur dioxide until all the dichromate was used up. This stage was further indicated by the development of a characteristic blue-green colour which was distinct from the bright emerald green of the earlier stages. All the stannous salts acted in the cold, but they contained varying quantities of nitrogen. Moreover, large quantities of these preparations had to be used because they had undergone partial oxidation to the stannic condition. Ferrous sulphate also acted in the cold, but considerable amounts of that chemical had to be used to ensure complete reduction of the dichromate. This resulted, naturally, in heavy precipitation of iron in the alkaline medium. There was violent bumping during distillation which could be prevented only by addition of small quantities of Devarda's alloy. Hydrogen peroxide was very effective in reducing the dichromate, but the analytical reagent used for that purpose contained some nitrogen.

Comparative efficiencies of different reducing agents in releasing combined nitrogen.—Samples (10 g.) of three different soils were digested in the manner previously outlined. The digests were treated with the different reducing agents, boiled to drive off excess of any acid vapour (such as sulphur dioxide) which may be present, cooled and finally distilled with excess of alkali. The results, after correction for the nitrogen contents of the reducing agents, have been presented in Table III.

There was general improvement in the estimate of total nitrogen when treatment with any of the above-mentioned reducing agents was followed by addition of zinc. The latter was added in the form of turnings (2 g.) and the digest then boiled for 15 mins. to complete the reduction (Table IV).

It may be noted, however, that in none of the cases was the result so high as that obtained by Kjeldahl 'wet' digestion.

The foregoing observations are highly significant. They show that mere reduction of dichromate is not sufficient to release all the nitrogen present in the digest. As already observed by Narayanayya and Subrahmanyan, even chromic salts can hold considerable quantities of nitrogen in combination, so that some special treatment will be needed to release all the nitrogen present in that form. The above results show that all reducing agents were not equally effective in that respect. The efficiencies of the different treatments also varied with the nature of the soil. These observa-

TABLE III.

Reducing agent used	Total Nitrogen in parts per million		
	Soil from		
	Bangalore	Kandy	Nilgiris
No reduction (control)	704	1269	1079
Oxalic acid (15 g.)	729	1328	1293
Sodium sulphite (10 g.)	729	1695	1750
Ferrous sulphate (15 g.)	708	..	..
Stannous chloride (15 g.)	722	1514	1622
Stannous sulphate (25 g.)	718	..	..
Hydrogen peroxide (10 c.c., 30 per cent.)	710	..	..
Value expected (Kjeldahl 'wet' digestion)	764	1769	1819

TABLE IV.

Treatment	Total Nitrogen in parts per million		
	Soil from		
	Bangalore	Kandy	Nilgiris
Oxalic acid (10 g.) +zinc (2 g.)	734	1639	1734
Stannous chloride (15 g.) +zinc (2 g.)	738	1676	1748
Sodium sulphite (10 g.) +zinc (2 g.)	744	1725	1781

tions would suggest one of the following :—(*a*) there are a number of compounds of chromium or mercury with nitrogen which requires varying degrees of reduction or (*b*) chromium or mercury forms only one nitrogenous compound which is attacked partially by some reducing agents and more fully by others. Subsequent reduction with zinc releases further quantities of nitrogen, if

any, from combination and, at the same time, reduces any nitrate which may be present in the medium. The precise mode of action of the reducing agents requires further elucidation. It may, nevertheless, be concluded from the above that to ensure the inclusion of all the different forms in the estimate of total nitrogen, the digest should be treated with the most effective combination of reducing agents which will, at the same time, yield the smallest blank for nitrogen. From this point of view, the combination of sulphite with zinc was found to be the most satisfactory among those so far tried. Further results on this aspect of the problem will be considered in a later section.

Order of addition of reagents.—With a view to finding whether this was of any importance in determining the efficiency of digestion, some experiments were carried out with three different types of soils (in 10 g. lots) adding the reagents, potassium dichromate (10 g.), water (20 c.c.) and sulphuric acid (40 c.c.), in the order mentioned in Table IV. In all the cases, the mixtures were heated gently in the early stages followed by boiling under reflux for 30 mins. The digests were reduced with sulphite and zinc and then distilled with excess of alkali in the usual way.

TABLE V.

Soil from	Total Nitrogen in parts per million			
	Order of Addition			Value expected (Kjeldahl 'wet' digestion)
	(1) $K_2Cr_2O_7$; (2) H_2O; (3) H_2SO_4	(1) H_2O; (2) $K_2Cr_2O_7$; (3) H_2SO_4	(1) H_2O; (2) H_2SO_4; (3) $K_2Cr_2O_7$	
Tindivanam	116	112	108	137
Jacobabad	383	372	375	394
Nandyal	280	271	275	297

It may be noted that in all the cases the values were lower than those obtained by 'wet' digestion.

Effect of conducting the digestion in two stages.—It was first considered probable that the loss of nitrogen might have been due to the decomposition of some of the immediate products of oxidation consequent on the steady rise of temperature to the boiling point (170°). Some experiments were carried out, therefore, adding the reagents in the order, dichromate, water and sulphuric acid and maintaining the reaction mixture at 100° for 30 mins.

before raising it to boil. The digestion was then conducted in the usual way. (Table VI).

TABLE VI.

Soil from	Total Nitrogen in parts per million	
	Expected (Kjeldahl ('wet' digestion)	Found
Bangalore	764	729
Belgaum	466	436
Tindivanam	137	95

The values were all distinctly lower than those expected. Pre-heating for longer periods at 100° led to further fall in the estimate of total nitrogen. It may be concluded, therefore, that such a procedure is not only unnecessary but also undesirable.

Effect of pre-heating with acid prior to addition of the oxidising agent.— Some experiments were next carried out, heating the mixtures of soil (10 g.) and acid (2 : 1, 60 c.c.) to 80° and the boiling temperature (170°) respectively and then adding the dichromate (5 g.). The digestion was then carried out in the usual way followed by reduction with sulphite and zinc. The results have been presented in Table VII.

TABLE VII.

Soil from	Total Nitrogen in parts per million			
		Pre-heating to		Expected (Kjeldahl 'wet' digestion)
	No pre-heating (control)	80°	Boiling	
Tanjore	779	786	793	793
Nilgiris	1722	1767	1818	1816
Kandy	1700	1715	1766	1764

There was distinct improvement on adding the oxidising agent to the hot mixture (80°) of soil and acid. When the same treatment was repeated with the boiling mixture, correct estimates (within limits of experimental error) were obtained.

Effect of adding the oxidising agent after boiling with acid for varying periods of time.—Samples (10 g.) of three different soils were treated with mercuric oxide (2 g.), water (20 c.c.) and acid (40 c.c.) and the mixtures raised to boil. One set of samples was treated with potassium dichromate (5 g.) immediately after commencement of boiling. The heating was then resumed and continued for a further period of 30 mins. Other sets were treated after 5, 10 and 20 minutes respectively after boiling and then digested for 30 mins. as before. The different digests were allowed to cool, diluted with water, reduced with sodium sulphite and zinc and finally distilled with excess of alkali (Table VIII).

TABLE VIII.

Soil from	Total Nitrogen in parts per million				
	Oxidising agent added after boiling for				Value expected (Kjeldahl 'wet' digestion)
	0 min. (Immediate)	5 mins.	10 mins.	20 mins.	
Chota Nagpur	209	209	207	212	209
Belgaum	466	468	468	466	466
Nandyal	293	293	295	295	295

Allowing for small experimental errors, it may be seen that the same values were obtained in all the cases. It may be concluded, therefore, that prolonged pre-boiling is unnecessary and that accurate results can be obtained by adding the oxidising agent even immediately after commencement of boiling.

Comparative efficiencies of different reducing agents.—The conditions for digestion having been standardised, attention was next directed to a comparison of the efficiencies of different methods of reduction in acid as well as alkaline media. Although the combination of sodium sulphite and zinc had proved highly useful, it was nevertheless realised that further improvement in the mode of reduction would be desirable. Thus, addition of sulphite had to be followed by boiling to drive off excess of sulphur dioxide, for, otherwise any acid vapour that may be present in the space above may pass over into the distillate and thus affect the titre value. Moreover, zinc had to be added only in the acid medium. Boiling was necessary to ensure complete reduction of nitrates and release of nitrogen combined with mercury or chromium. Although only small quantities of zinc (2 g.) were used, the action was

rendered rather slow by the presence of mercury salts which, as already explained, formed protective coats of hydride around unattacked zinc. These were not serious difficulties, but it was still considered desirable to avoid boiling in acid medium. Excess of chromic acid could be reduced in the cold and the reduction of nitrates and other complex forms, completed in alkaline medium, during distillation. A number of trials were accordingly carried out with a variety of soils employing different combinations of reducing agents. The results obtained with one soil have been presented in Table IX.

Samples (10 g.) of a specimen of Bangalore soil were digested according to the improved procedure and, after dilution, treated with different reducing agents followed by boiling. The digests were cooled and then distilled with excess of alkali in the usual way. The nitrogen content of the different reducing agents were determined separately. Alkali equivalent of total nitrogen as found by 'wet' Kjeldahl digestion was 17·25 c.c. (850 p.p.m.).

It may be mentioned that although two samples of zinc (dust and turnings) were first tried, only the turnings were used in the subsequent experiments where zinc was used in combination with other reducing agents. Potassium sulphide was generally added as a strongly alkaline solution, having been previously dissolved in the alkali to be used for distillation. Sodium sulphite, though highly reactive in acid medium, was quite ineffective in presence of excess of alkali. Reduction with ferrous sulphate was followed by heavy precipitation in alkaline medium, but this disadvantage can be overcome by addition of small quantities of Devarda's alloy. Distillation with more than about 1 g. of Devarda's alloy led to considerable frothing, the alkali being nearly always carried over with the spray. In consequence, the duplicates did not generally agree. The tendency to spray was greatly retarded in presence of tin or lead salts in the medium. This was due to the reduction of those salts to the metallic condition followed by the formation of the corresponding hydrides, which formed partially protective coats around unattacked Devarda's alloy. The vigour of the interaction with alkali was consequently retarded and the reduction proceeded smoothly, though somewhat more slowly, than might otherwise have been the case. This observation is of considerable practical significance and may be utilised with advantage when having to conduct reductions with Devarda's alloy or aluminium in alkaline media. Three or four brands of stannous salts were tried but they were generally impure and contained varying proportions of nitrogen. Moreover, with the exception of a few samples of analytical reagents in sealed bottles, all the others contained considerable amounts of the corresponding stannic salts having undergone spontaneous oxidation in presence of air. A similar observation was also made in regard to titanous chloride which was, in consequence, found to be unsuitable for the reduction.

TABLE IX.

Reducing agent employed	Alkali equivalent of nitrogen (as c.c. of 0·0352 N alkali)		Total nitrogen (in p.p.m.) after correction for blank
	Total nitrogen (without correction for blank)	Nitrogen content of reducing agent (blank)	
No reduction (control) ..	15.8, 15.95	..	783
Oxalic acid (10 g.) ..	16.4, 16.2	Nil.	803
Oxalic acid (10 g.) and zinc (2 g.)	16.5, 16.6	0.15	808
Oxalic acid (10 g.) zinc (2 g.) followed by potassium sulphide (5 g.) in alkaline medium	16.65, 16.75	0.15	811
Oxalic acid (10 g.) followed by Devarda's alloy (2 g.) in alkaline medium ..	16.2, 16.1	Nil.	796
Zinc dust (10 g.) ..	23.25, 23.45	6.6 (?)	825 (?)
Zinc turnings (pure, 10 g.) ..	17.3, 17.4	0.6	821
Zinc dust (10 g.) followed by K_2S (5 g.) in alkaline medium..	23.35, 23.40	6.6 (?)	827 (?)
Sodium sulphite (10 g.) ..	16.5, 16.7	Nil.	818
Na_2SO_3 (10 g.) followed by K_2S (5 g.) in alkaline medium ..	16.7, 16.9	Nil.	828
Na_2SO_3 (10 g.) and Zn turnings (2 g.) in acid medium ..	17.4, 17.45	0.20	850
Na_2SO_3 (10 g.) followed by Devarda's alloy (2 g.) in alkaline medium ..	16.8, 16.7	Nil.	826
Na_2SO_3 (10 g.) and Devarda's alloy (2 g.) both in alkaline medium	Ineffective—Sodium sulphite does not reduce in alkaline medium.		
K_2S (10 g.) followed by zinc (2 g.) in acid medium ..	16.8, 16.7	0.15	813

TABLE IX—(*Contd.*)

Reducing agent employed	Alkali equivalent of nitrogen (as c.c. of 0·0352 N alkali)		Total nitrogen (in p.p.m.) after correction for blank
	Total nitrogen (without correction for blank)	Nitrogen content of reducing agent (blank)	
K_2S (10 g.) followed by Devarda's alloy (2 g.) in alkaline medium.	16.0, 15.95	Nil.	789
Reduced iron (10 g.) ..	18.05, 18.2	1.45	822
Reduced iron pre-boiled with alkali (10 g.) ..	17.65, 17.75	0.9	830
$FeSO_4$ (15 g.) in acid medium ..	16.95, 17.0	0.65	803
$FeSO_4$ (15 g.) and Zn (2 g.) in acid medium ..	17.25, 17.30	0.80	813
$SnCl_2$ (15 g.) in acid medium ..	16.4, 16.3	Nil.	805
$SnSO_4$ (15 g.) and zinc in acid medium ..	17.1, 17.2	0.15	838
$SnCl_2$ (15 g.) in acid medium and Devarda's alloy in alkaline medium ..	16.8, 16.8	Nil.	828

It may be seen from the results, that the most accurate results were obtained by using a mixture of sodium sulphite and zinc for the reduction. Some of the other combinations such as oxalic acid and zinc or stannous chloride and zinc, were, in some respects, easier to deal with but they generally yielded slightly lower estimates. In this connection, the results obtained with two of the soils previously examined would be of some interest (Table X).

The foregoing observations would suggest that although sodium sulphite was most potent in releasing combined nitrogen than either stannous chloride or oxalic acid, the difference could be largely made up by supplementing the reduction with zinc. It was not clear, however, whether addition of larger quantities of zinc would further improve the estimate of total nitrogen. With this in view, some experiments were carried out in which, after reduction with oxalic acid, parallel samples were treated with different quantities of zinc and boiled in the usual way. The results are presented in Table XI.

TABLE X.*

Soil from	Total Nitrogen in parts per million after reduction with		
	Sodium sulphite (10 g.) and zinc (2 g.)	Oxalic acid (10 g.) and zinc (2 g.)	Stannous chloride (15 g.) and zinc (2 g.)
Kandy	1767	1653	1700
Nilgiris	1821	1757	1772
Bangalore	764	737	742

* These results are distinct from those in Table IV. In the latter case, mixture of soil, dichromate and acid were heated together.

TABLE XI.

Soil from	Total Nitrogen in parts per million after reduction with				Total nitrogen expected (Kjeldahl "wet" digestion)
	Oxalic acid (10 g.) alone	Oxalic acid (10 g.) and zinc (2 g.)*	Oxalic acid (10 g.) and zinc (3 g.)*	Oxalic acid (10 g.) and zinc (4 g.)*	
Nilgiris ..	1343	1757	1765	1770	1816
Kandy ..	1377	1653	1653	1681	1764

* After correction for nitrogen in zinc.

It may be noted that although increasing quantities of zinc led to slight improvement in the estimate of total nitrogen, the values, thus obtained, were not so high as those by 'wet' digestion. When combined with sodium sulphite, however, even small quantities of zinc were quite effective, and as may be seen from the foregoing results, yielded correct estimates of total nitrogen in every case.

Inclusion of nitrate in the estimate of total nitrogen.—In the previous communication, a special procedure was suggested for the inclusion of nitrate in the estimate of total nitrogen. That would, no doubt, be needed if the quantity of nitrate is very large, but most soils do not contain more than traces of that form of nitrogen. Moreover, it is not probable that any nitrate will be lost when the digestion is carried out under reflux, so that if the subsequent procedure is adequately controlled, the entire quantity may be included in the estimate of total nitrogen. It may be reasonably expected that treatment with zinc in acid medium will help to reduce the moderate

quantities of nitrate which may be ordinarily present in the soil ; that in the case of soils containing useful amounts of nitrate, the method of oxidative digestion should give higher values than the Kjeldahl method. If the latter is modified, however, to include nitrates, then the two estimates should agree. Such was indeed the case as may be seen from the results obtained with some specimens of soils (Table XII).

TABLE XII.

Soil from	Total Nitrogen in parts per million by		
	Kjeldahl 'wet' digestion*	Wet digestion to include nitrate †	Oxidative digestion
Punjab	527	545	543
Sindh (*Kalar*) ..	513	522	524
Ahmedabad	372	394	397
Dacca	669	700	702

* Soil (10 g.) digested with water (20 c.c.) and sulphuric acid (40 c.c.), K_2SO_4 (10 g.) added at the fume stage.

† The same as above together with ferrous sulphate (2 g.) added to the digesting mixture. (Sreenivasan, *J. Ind. Inst. Sci.*, 1935, **18A**, 25).

Although the specimens previously examined did not show any appreciable difference between the results of Kjeldahl 'wet' and oxidative digestions, the present set brought into relief a further aspect of the distinct superiority of the latter over the former. According to the usual Kjeldahl method any nitrate which may be present in the soil will be lost : special pre-treatment of the soil will be needed if the nitrate is to be included in the estimate of total nitrogen. On the other hand, the procedure adopted for oxidative digestion naturally includes nitrates and is, in consequence, more advantageous than the other methods.

Effect of addition of nitrate to soil.—Sreenivasan (*loc. cit.*) has already shown that zinc is only moderately effective in reducing nitrates in acid medium. It was considered desirable therefore to determine the extent to which the use of that reagent will help to include nitrates in the estimate of total nitrogen. To samples (10 g.) of two different soils, known quantities of nitrate were added. The mixtures were digested under reflux in the usual way after which they were reduced with the same quantities of sodium sulphite (10 g.) and zinc (2 g.) in each case. The results, which have been

given in Table XIII show that at least upto 50 parts per million of the nitrates (excluding any that may be already present in the soil) can be included in the estimate of total nitrogen. The average soil in any part of the world, rarely ever contains more than 50 p.p.m. of nitric nitrogen. The tropical soils generally contain very much less. It may not therefore be necessary to adopt any further modification to include nitrates.

TABLE XIII.

Soil from	Total Nitrogen in parts per million						
	Soil alone	Soil + nitrate (10 p.p.m.)	Soil + nitrate (20 p.p.m.)	Soil + nitrate (30 p.p.m.)	Soil + nitrate (40 p.p.m.)	Soil + nitrate (50 p.p.m.)	Soil + nitrate 100 p.p.m.
Nagpur–(Black cotton) ..	422	432	441	452	460	470	474
Mandalay ..	340	349	359	371	381	386	390

There are, no doubt, a few exceptional conditions in which special treatment may be needed. It is probable that in some cases fairly large quantities of soil (20–25 g.) may have to be taken for digestion. It may also occasionally happen that the soil is unusually rich in nitrates or has been recently fertilised with that form of nitrogen. In such cases more vigorous reduction than that provided by zinc alone may be needed. Reduced iron is known to be more efficient (Olsen, 1927), but generally large quantities of that reagent are required. Moreover, the nitrogen content of iron, as also the heavy precipitation during distillation, may militate against its use as a reducing agent. It would therefore appear to be preferable to use Devarda's alloy either before digestion, as outlined in the previous part, or together with alkali during distillation. Of the two, the former procedure is to be preferred because (*a*) it requires less attention and (*b*) there is no danger of any alkali being carried over into the distillate.

Some of the factors determining the efficiency of oxidative digestion : Loss of nitrogen consequent on adding dichromate to cold mixtures of ammonium salts and acid.—Attention has already been drawn to the fact that low estimates of nitrogen were invariably obtained when dichromate was added to a cold or even fairly hot mixture of soil and acid. It was not clear, however, as to whether the difference was due to loss of nitrogen in gaseous form or to its retention in the digest in some highly resistant form. With a view to throwing some light on this aspect of the problem, some experiments were carried out adding dichromate (5 g.) to dilute solutions of ammonium sulphate in sulphuric acid (2 : 1) and heating the mixtures to gentle boil. After boiling for 30 mins., the mixtures were cooled, reduced with sulphite and zinc and

distilled with excess of alkali in the usual way. In another experiment, a solution of ammonium dichromate was heated with excess of potassium dichromate while, in a third one, ammonium dichromate alone was boiled with 2 : 1 sulphuric acid. The results which have been presented in Table XIV show that there was very nearly complete recovery in the case of ammonium

TABLE XIV.

Treatment	Alkali equivalent of nitrogen (in terms of c.c. of 0·0352 N alkali)		Average recovery per cent.
	Expected	Found	
$(NH_4)_2SO_4+K_2Cr_2O_7$ (5 g.)+ H_2SO_4 (60 c.c., 2 : 1)	11·80	11·65, 11·60, 11·70, 11·45	98·3
$(NH_4)_2Cr_2O_7+K_2Cr_2O_7$ (5 g.)+ H_2SO_4 (60 c.c., 2 : 1)	12·85	12·10, 12·15	94·4
$(NH_4)_2Cr_2O_7+H_2SO_4$ (60 c.c., 2 : 1)	233·5	231·8, 232·1, 232·9, 231·7	99·4

dichromate alone whereas, in the others, small amounts of nitrogen were lost. The latter observations are in agreement with the findings of Shewan (1935). The loss of nitrogen was completely avoided, however, by adding potassium dichromate to a boiling mixture of ammonium sulphate and acid (2 : 1). (The results actually obtained were 11·80, 11·85, 11·80 and 11·80 respectively against an expected value of 11·80.) The loss of nitrogen consequent on heating mixtures of ammonium sulphate and dichromate in acid medium may have been due to either or both of the following causes : (1) A part of the ammoniacal nitrogen may have been directly oxidised to the elementary condition. (2) Small quantities of ammonium dichromate, first formed in the cold, may have decomposed on heating, yielding elementary nitrogen. If the first explanation is correct, then there should have been some loss of nitrogen even on adding the dichromate to the boiling mixture of ammonium salt and acid. Moreover, other oxidising agents should also cause similar loss of nitrogen. The previous observations of Sreenivasan with hydrogen peroxide and of other workers with perchloric acid would show that there is no loss of nitrogen in those cases. The second explanation also, if taken as such, is comparatively weak. Pure ammonium dichromate itself—at any rate in such small quantities as are present in Kjeldahl digests—undergoes very little loss when heated with sulphuric acid. It is converted, more or less quantitatively, into ammonium sulphate. On

the other hand, the large excess of potassium dichromate (or the resulting chromic acid) in the digesting mixture would tend partly to reverse the reaction.

$$(NH_4)_2SO_4 + H_2Cr_2O_7 \rightleftarrows (NH_4)_2Cr_2O_7 + H_2SO_4$$

The quantity of chromic acid present in solution at any time may be small, but there is always excess of the precipitated trioxide, a part of which will pass into solution and make up for the saturation. In this manner, small quantities of ammonium dichromate may persist in the medium during heating and may decompose at some temperature below the boiling point of 2 : 1 sulphuric acid. If, on the other hand, the dichromate (or chromic acid) is added to the boiling, acid solution, then ammonium dichromate may not be formed at all, and loss of nitrogen may be entirely avoided. To verify this the following pieces of evidence are required : (*a*) that ammonium dichromate undergoes spontaneous decomposition at some temperature below the boiling point of 2 : 1 acid and (*b*) that nitrogen is lost from mixtures of ammonium sulphate, and chromic acid (in excess) at the same stage as the one at which ammonium dichromate decomposes.

With a view to throwing some light on these and other aspects of the problem, the following experiments were carried out.

Effect of heating ammonium sulphate with other oxidising agents in acid medium.—To aliquots (10 c.c.) of an aqueous solution of ammonium sulphate, sodium bismuthate (5 g.), potassium persulphate (2 g.) and hydrogen peroxide (10 c.c. ; 30 per cent.) respectively were added and the mixtures treated with 10 c.c. each of concentrated sulphuric acid. The mixtures were then heated under reflux for 30 mins. after which they were distilled with excess of alkali in the usual way. Since bismuth was found to hold small quantities of nitrogen, the digests obtained after treatment with bismuthate were treated with excess of potassium sulphide together with alkali.

There was complete recovery in all the cases thereby showing that the presence of oxidising agents did not lead to any loss of nitrogen.

The above experiment was not repeated with permanganate because it is known that ammonium permanganate undergoes spontaneous decomposition on heating. Consequently, even if there was any loss of nitrogen, it would not have been possible to state whether it was due to direct oxidation or to intermediary formation of the ammonium salt.

The stage at which ammonium dichromate undergoes spontaneous decomposition in acid medium.—It is well known that the decomposition of ammonium dichromate does not start at any particular temperature. Our observations showed that even with the most careful heating, the decomposi-

tion of solid ammonium dichromate started sometimes in the vicinity of 130° and sometimes above 140°. When the dichromate was suspended in sulphuric acid, the decomposition did not generally commence until a temperature of 135° was reached. In some cases it began at a slightly higher temperature, but in no case did it exceed 145°. The decomposition was characterised by the entire medium turning blue green. There was also considerable evolution of gas. This was distinct from the rather slow decomposition of potassium dichromate with which control experiments were carried out.

The stage at which nitrogen is lost when mixtures of ammonium sulphate and dichromate are heated.—Aliquots (20 c.c.) of a standard solution of ammonium sulphate were treated with potassium dichromate (5 g.) and sulphuric acid (40 c.c.) and the mixtures heated to different temperatures. They were then treated with sodium sulphite (10 g.) and zinc (2 g.) and distilled with excess of alkali. The results (Table XV) show that the loss was significant at 130°, and quite pronounced at 150°. There was no further loss at 170°. These observations would show that the loss of nitrogen occurred between 130° and 150°. This range would correspond to the decomposition of ammonium dichromate.

TABLE XV.

Temperature	40°	60°	80°	100°	120°	130°	140°	150°	170°
$(NH_4)_2SO_4$ present in terms of c.c. 0·0352 N alkali	23·6	23·6	23·55	23·5	23·45	23·25	23·05	22·9	22·9

Value expected = 23·6 c.c.

It would appear, therefore, that, with the steady rise in temperature, the ammonium dichromate formed in the medium underwent partial decomposition. The extent of decomposition was comparatively small because of the presence of acid which combined preferentially with ammonia. The loss of nitrogen was nevertheless quite significant.

Effect of adding dichromate at different temperatures.—The results of the previous experiment would suggest that if the dichromate is added in the cold or at any temperature below 150° there would be some loss of nitrogen. If, on the other hand, the digesting mixture is heated above this point, the intermediary ammonium salt will not be formed and the loss of nitrogen will be avoided. This conclusion is partly supported by the fact that there is no loss of nitrogen on adding the dichromate to the boiling mixture of ammonium salt and acid (temp., 170°). With a view to obtaining further

quantitative evidence, aliquots (20 c.c.) of a standard solution of ammonium salt were treated with concentrated sulphuric acid (40 c.c.) and raised to different temperatures. They were then treated with potassium dichromate (5 g.) and raised to boil. After boiling for 30 mins., the mixtures were reduced with sulphite and zinc and then distilled with excess of alkali. The results have been presented in Table XVI.

TABLE XVI.

Temperature	120°	130°	135°	140°	145°	150°	160°	170°
N recovered in terms of c.c. of 0·0352 N alkali	22·3	22·4	22·8	22·95	22·95	23·05	23·2	23·2

Value expected = 23·2 c.c.

It may be seen from the above that there was no loss of nitrogen when the dichromate was added at temperatures above 160°. These observations lend further support to the intermediary formation of ammonium dichromate at low temperatures. They also show that ammonium dichromate is not formed at higher temperatures so that the attendant loss of nitrogen is entirely avoided.

Effect of re-heating chromic acid digest of soil.—In view of the presence of ammonium sulphate and unused chromic acid in the final digest, it was considered probable that re-heating of such a mixture would cause some loss of nitrogen. Some experiments were accordingly carried out with a specimen of Bangalore soil which was first digested for 30 mins., by pre-heating to boiling followed by addition of dichromate. One set of digests was then reduced with sulphite and zinc in the usual way. The other was allowed to cool and then heated slowly to boiling. They were then reduced and distilled. The estimates of nitrogen thus obtained were as follows:—without re-heating (control), 603 p.p.m. (average value) ; with re-heating, 583.

In addition to supporting the previous findings, these observations are also of some practical interest. They show that after completion of digestion, the residue should not be re-heated. It is not also advisable to stop the heating before completion of digestion and then to resume it at a later stage.

Other evidence in support of the intermediary formation of ammonium dichromate will be considered elsewhere.

Kinetics of digestion when the oxidising agent is added before or after boiling.—With a view to determining the progress of digestion under the two sets of conditions, parallel samples (10 g.) of three different specimens of soils were treated with the same quantities of mercuric oxide (2 g.), water

(20 c.c.), sulphuric acid (40 c.c.) and dichromate and the digestion conducted in the usual way. In one set, the dichromate (5 g.) was added together with the other reagents, whereas, in the other, it was added only after the mixtures came to boil. The digestion of parallel samples was stopped at intervals of 5 mins. commencing from the moment of boiling. The digests were treated with sodium sulphite (10 g.) and zinc (2 g.) and then distilled with excess of alkali. The results have been presented in Tables XVII and XVIII.

TABLE XVII.

Dichromate added in the cold.

Soil from	Total nitrogen in parts per million as estimated after (time in mins.)						Value expected (control)
	0	5	10	15	25	30	
Cuttack	306	343	345	377	382	382	402
Gorakhpur	774	798	811	808	811	813	850
Gaya	325	387	432	438	438	440	451

TABLE XVIII.

Dichromate added after commencement of boiling.

Soil from	Total nitrogen in parts per million as estimated after (time in mins.)						Value expected (control)
	0	5	10	15	25	30	
Cuttack	392	394	397	399	402	402	402
Gorakhpur	806	821	831	850	850	850	850
Gaya	417	429	446	448	451	451	451
Nagpur	404	414	421	424	424	424	422

It may be noted that in both the sets of experiments, the digestion proceeded very rapidly, the estimates of total nitrogen reaching steady values in under 25 minutes. In the first series (Table XVII) the values were distinctly lower than those expected whereas in the latter (Table XVIII) they agreed closely. This may be traced to the difference in the starting values (0 min.) which were distinctly higher when the oxidising agent was added to the boiling mixture than when it was added in the cold. Since the progress of digestion was more or less the same after commencement of boiling, it may be inferred that in the first series (Table XVII) the loss had occurred

even before that stage. These observations thus lend support to the previous findings and show that the loss of nitrogen was due to the intermediary formation of ammonium dichromate.

The results presented in Table XVIII show that the moment the oxidising agent was added to the boiling mixture of soil and acid, over 90 per cent. of the nitrogen was digested. The remaining part was also rapidly attacked and the digestion was complete within 15 mins. after commencement. Although this observation would suggest further shortening of the period of digestion, it may, nevertheless, be desirable, in routine practice, to continue the boiling for at least 30 mins., so that even the most resistant forms may be included in the estimate of total nitrogen.

Rate of digestion of urea.—Attention has already been drawn to the difficulty in digesting urea by the oxidative method (Shewan, *loc. cit.*; Narayanayya and Subrahmanyan, *loc. cit.*). It was, nevertheless, considered probable that by adopting the improved procedure, the need for pre-boiling with acid may be avoided. Some experiments were accordingly carried out treating aliquots of a standard solution of urea with sulphuric acid (in the proportion 2 : 1) and adding dichromate after the mixtures came to boil. At intervals of 5 mins., representative specimens of digests were removed, and, after reduction with sulphite and zinc, distilled with excess of alkali. In another set of experiments, representative specimens (10 g. each) of soils were mixed with urea in solution and then digested in the same manner as in the previous experiment. The results have been given in Table XIX.

TABLE XIX.

Material digested	Alkali equivalent of nitrogen after digestion for (as c.c. of 0·0352 N alkali) (time in mins.)						Value expected (control)
	0	5	10	15	20	30	
Urea (in solution, 10 c.c.)	5·25	5·45	5·55	5·55	5·55	5·55	5·55
Soil (from Cuttack)+ urea (in solution, 10 c.c.)	13·20	13·50	13·55	13·17	13·70	13·70	13·70
Soil (from Gorakhpur) +urea (in solution, 10 c.c.)	21·90	22·20	22·50	22·60	22·75	22·75	22·75

It may be noted that the digestion proceeded quantitatively in all the cases. The digestion of urea alone was complete in 10 mins., but when admixed with soil, a few more minutes were taken. As previously observed

in the case of soil, more than 90 per cent. of the digestion was complete immediately after addition of oxidising agent.

As already observed by Narayanayya and Subrahmanyan, urea can be easily digested by 2 : 1 acid alone. It is also rapidly decomposed by alkali yielding ammonia. These observations show that urea, by itself, does not require any oxidative digestion and will yield correct estimates even without treatment with dichromate. The loss of nitrogen consequent on heating mixtures of urea, dichromate and acid should therefore be traced to the intermediary formation of ammonium dichromate. As in the other cases, the formation of the intermediate compound and the attendant loss of nitrogen can be avoided by adding potassium dichromate to the boiling mixture of urea and acid.

Trials with some representative specimens of soils.—With a view to determining whether the method of oxidative digestion can be applied successfully to all types of soils, trials were carried out with representative specimens from India, Burma and Ceylon. In all the cases, air dry specimens (10 g.) passing the 100-mesh sieve were used. All the samples were treated with mercuric oxide (2 g.) to eliminate any possible error due to the presence of halides. Water (20 c.c.) and sulphuric acid (40 c.c.) were then added in succession and after the mixtures came to boil, they were treated with 5 g. each of dichromate. The digestions were continued for 30 mins. after which the digests were diluted with about 300 c.c. of water and then treated with sodium sulphite (10 g.) and zinc (2 g.). The mixtures were then boiled for about 15 mins. after which they were distilled with excess of alkali in the usual way. The results have been presented in Table XX.

It may be seen from Table XX that there was very close agreement between the two sets of values. In many cases, the same estimates were obtained, whereas, in others, the difference was less than what would be accounted by 0·1 c.c. of the alkali used for back titrations.

Nitrogen contents of some biological materials.—The estimations were next extended to biological materials some of which had offered considerable difficulty in the previous study. In all the cases, the oxidising agent was added to the boiling acid mixture after which the digestion was continued for only 30 mins. The digests were all reduced with sulphite and zinc and then distilled with excess of alkali. The results have been given in Table XXI.

There was close agreement in all the cases. Even cyanamide, which offered considerable difficulty in the previous study, was readily digested on adding the oxidising agent to the boiling mixture. Yeast is another biological material which is not easily digested. In the foregoing experiment,

TABLE XX.

Description of soil	Total nitrogen in parts per million	
	Expected*	Found
Sind (Willingdon Cattle Farm)–*kalar*–green manured ..	520	522
Tanjore (Madras)–deltaic area–alluvial–surface ..	793	793
Nandyal (Madras)–red clay–surface	296	296
Cuttack (Orissa)–upland–surface	399	402
Gaya (S. Bihar)–lowland, paddy–surface	451	451
Jacobabad–Govt. wheat farm–surface	397	394
Tindivanam (Madras)–dryland–surface	138	138
Gorakhpur–lowland–surface	848	850
N. Bihar–paddyland–surface	643	640
Devarshola (Nilgiris)–estate–surface	1819	1821
Kandy (Ceylon)–paddyland–sub-soil	1769	1767
Dharwar (Bombay)–old area–sub-soil	537	537
Sholapur–heavy black–surface	244	242
Jaffna (Ceylon)–sub-soil	397	399
Dacca–highland–cultivated–sub-soil	700	702
Chirakkal–sandy loam–surface	315	315
Travancore–alluvial–surface	1972	1973
Sind–uncultivated–surface	352	350
Bangalore–sandy loam–surface	850	850
Nagpur–black cotton–surface	420	422
Mandalay (Burma)–paddy–sub-soil	339	340

TABLE XX—(*Contd.*)

Description of soil	Total nitrogen in parts per million	
	Expected*	Found
Punjab–Barani area–sub-soil	542	542
Ahmedabad–Kambha village–sub-soil	394	397
Chota Nagpur–laterite–sub-soil	209	209
Belgaum–cultivated–sub-soil	466	466

* Including Nitrates (Sreenivasan, *J. Ind. Inst. Sci.*, 1935, **18A**, 25).

TABLE XXI.

Material	Total Nitrogen per cent.	
	Expected	Found
Cyanamide	16·51	16·50
Dried blood	12·38	12·41
Groundnut cake ..	7·72	7·68
Lantana leaf	2·80	2·81
Paddy husk	0·27	0·27
Ragi straw	0·52	0·52
Yeast	5·65	5·65

the Kjeldahl digestion was possible only after pre-treatment with alkali followed by prolonged digestion with concentrated acid (S. Rajagopal, private communication). On the other hand, oxidative digestion was comparatively simple and proceeded to completion, as rapidly as in any other case. Paddy husk is another material known to be highly resistant to Kjeldahl digestion : but even that was easily digested according to the new method.

Is it always necessary to use a condenser during digestion ?—Attention has already been drawn to the fact that, at any rate in the case of soils, only traces of nitric acid are lost by volatilisation during oxidative digestion.

Since most soils are naturally poor in nitrates and since there is always a useful quantity of water in the digesting medium, it was considered probable that, even without any cooling arrangement, only traces of nitrates would be ordinarily lost by volatilisation. With a view to determining whether the condenser (air or water-cooled) can be dispensed with in routine practice, some experiments were carried out in which parallel samples of the same soils were digested with and without cooling. The details of digestion, reduction and distillation were the same as those already outlined. The results which have been presented in Table XXII show that although, in

TABLE XXII.

Soil from	Total Nitrogen in parts per million as estimated after digestion	
	With condenser	Without condenser
Nandyal ..	296	293
Tindivanam ..	138	133
Gorakhpur ..	850	835

two cases, very nearly the same estimates were obtained, the third one yielded distinctly lower values when the condenser was not used. This was traced to the presence of useful quantities of nitrate in the original soil itself. In view of this observation, it was considered desirable to use the condenser as an item of routine procedure. The air-condenser, if sufficiently long, is as efficient as the water-cooled one. It requires no attention, involves no extra expenditure and can be easily fitted to the digesting flask. It should, therefore, be preferred to the water-cooled condenser.

Use of chromic acid in place of dichromate.—The use of dichromate, though satisfactory in many respects, is attended by certain disadvantages. In ordinary practice it has to be weighed out (though only approximately) and has to be introduced into the hot boiling mixture after momentarily removing the condenser. This procedure, though fairly rapid, is rather inconvenient and should, if possible, be avoided. Some experiments were, therefore, carried out using aqueous solutions of chromic acid (CrO_3) as the oxidising agent and comparing the values with those obtained with dichromate. The procedure adopted in the case of chromic acid was to add a saturated aqueous solution (5 c.c. for every 10 g. of soil) directly through the condenser as soon as the soil-acid mixture came to vigorous boil. The results which

have been presented in Table XXIII show that the same results were obtained in both the sets of experiments. Aqueous solution of chromic acid being more convenient to handle, should be preferred to solid dichromate.

TABLE XXIII.

Soil from	Total Nitrogen in parts per million after oxidative digestion with	
	Solid dichromate	Chromic acid (aqueous solution)
Bangalore ..	850	848
Kandy ..	1769	1767
Dharwar ..	537	537
Sholapur ..	244	244
Jaffna ..	399	396
Chirakkal ..	315	314
Travancore ..	1972	1969

When using the aqueous solution it would be useful to reduce the volume of water that is first added by that of the solution of chromic acid. This procedure is suggested so as to maintain the proportion of sulphuric acid to water in the neighbourhood of 2 : 1.

It has been found that commercial brands of chromic anhydride generally contain minute quantities of nitric acid. When using that chemical, it would be desirable, therefore, to perform control experiments and apply the necessary correction.

Proportion of acid to water.—It was found previously (Narayanayya and Subrahmanyan, *loc. cit.*) that a proportion of 2 : 1 between sulphuric acid and water was most suited to ensure rapid and complete digestion of organic nitrogen. In view of the later improvements in regard to procedure, it was considered desirable to determine whether the proportion of acid to water can be reduced with advantage.

Some experiments were carried out, therefore, with parallel samples of two soils using the other reagents in the same proportions as in the previous experiments but only altering the proportion of sulphuric acid to water so as to correspond to 1 : 2, 1 : 1 and 2 : 1 respectively. The digestions were stopped at the end of 30 mins. in each case. The results, which have been

given in Table XXIV show that correct estimates were obtained after digestion for 30 mins. only when the proportion of acid to water was as 2 : 1. At the ratio 1 : 1, the values tended to approach the expected ones, while at 1 : 2 they were very much lower.

TABLE XXIV.

Soil from	Total Nitrogen in parts per million			Value expected
	Proportion of acid to water			
	1: 2	1: 1	2: 1	
Sindh ..	219	318	352	352
Bangalore ..	633	836	850	850

With a view to determining whether the values obtained at the ratio 1 : 1 can be further improved, the period of digestion was increased to 60 minutes in the case of two soils. The results (Table XXV) showed, however, that the values did not show any appreciable improvement.

TABLE XXV.

Soil from	Total Nitrogen in parts per million				Value expected
	Period of digestion in minutes				
	30	40	50	60	
Bangalore ..	836	831	833	828	850
Nagpur (Black Cotton) ..	402	396	407	411	422

The above results were first inexplicable, but subsequent observations showed that the boiling temperature of soil suspension in 1 : 1 acid (by volume) was 140°, which comes within the range of decomposition of ammonium dichromate (*vide supra*). Since the boiling mixture was refluxed, the temperature remained more or less constant and consequently there was some loss of nitrogen. A similar argument would apply, even more strongly, to the suspension in 1 : 2 acid which boiled at 115°. On the other hand, the boiling temperature (170°) of the suspension in 2 : 1 acid was well above the decomposition point, so that when the dichromate or chromic acid (in small instalments) was added to the boiling mixture, there was no scope for the formation of ammonium dichromate as an intermediate product and, consequently, there was no loss of nitrogen.

Effect of fineness of division on the efficiency of digestion.—It has been shown by Subrahmanyan, Narayanayya and Bhagvat (1934) as also by later workers, that wet combustion of soil will yield accurate estimates of carbon only when the soil is ground to a very fine state of division (passing the 100-mesh sieve). With a view to determining whether a similar condition is necessary to obtain accurate estimates of total nitrogen as well, some experiments were carried out with specimens of two different soils, grinding them to varying degrees of fineness and then digesting them (10 g. each) with dichromate (5 g.) and sulphuric acid (60 c.c., 2 : 1) in the usual way. The results (Table XXVI) showed that the fineness of division did not make any appreciable difference to the accuracy of the estimate of total nitrogen.

TABLE XXVI.

Soil from	Total nitrogen in p.p.m. when the soil is ground to pass (in meshes to the inch)				Value expected as p.p.m.
	30–40	40–60	60–90	Above 90	
Bangalore ..	601	603	603	601	603
Tellichery (Red sandy loam) ..	418	414	416	414	416

Efficiency of digestion at different proportions of soil to acid.—If sufficient quantities of material are available, it would be generally desirable to digest as large a quantity as possible, using the minimum quantity of acid for the purpose. With this in view, some experiments were carried out maintaining the quantities of water (20 c.c.), acid (40 c.c.) and dichromate (10 g.) the same, but only increasing that of the soil. The results (Table XXVII) show that upto 15 g., the digestion proceeded quantitatively. With further increase in quantity, however, there was distinct fall in the efficiency of digestion.

TABLE XXVII.

Soil from	Total Nitrogen in p.p.m. on digesting (Wt. in grams)					Value expected (in p.p.m.)
	5	10	15	20	25	
Bangalore ..	601	603	603	593	581	603

In this connection, it may be mentioned that when all the components of the digesting mixture are increased in the same proportion, then accurate estimates of total nitrogen may be obtained irrespective of the quantity of

soil taken. Our observations have shown that using the distilling flask (cap. 1,500 c.c.) itself for the digestion, 25-30 g. of soil can be easily digested and distilled in the usual way. Even larger quantities can be efficiently digested, but considerable amounts of acid and alkali will be required. The distillation will also be rendered difficult. In ordinary practice, quite accurate results can be obtained by digesting in 10 g. lots and it will be rarely necessary to digest more than 15 g. at a time.

Comparative efficiencies of digestion by different methods.—The several methods now in vogue for the estimation of nitrogen by acid digestion may be classified, under the following main heads.—(1) Those involving 'dry' digestion with concentrated sulphuric acid. Such methods may or may not include additional procedure for inclusion of nitrates. This type of digestion is still most extensively adopted and forms the basis of ' official ' methods recognised in different parts of the world. (2) ' Wet ' digestion involving preliminary treatment with water or dilute sulphuric acid, but otherwise similar to the first group. The advantages of this type of digestion are now being steadily realised. (3) Methods involving pre-treatment with hydrogen peroxide, perchloric acid or other oxidising agent combined with dilute sulphuric acid. These require special modification to include nitrates. They are quite rapid but do not eliminate the emission of fumes. (4) Oxidative digestion involving vigorous oxidation in a strongly acid medium. As already explained, this type of digestion is fumeless and includes nitrates in the estimate of total nitrogen. With a view to comparing the relative merits of these four types, representative samples of two soils (both containing only traces of nitrates) were digested according to (1) the Kjeldhal method (*Chemists' Year Book*, 1935) ; (2) the 'wet' method as recommended by Bal (1925) ; (3) Sreenivasan and Subrahmanyan (1933) using hydrogen peroxide as the oxidising agent ; and (4) the method of oxidative digestion as developed in the present paper. The digestion was stopped at definite intervals after commencement of boiling and the digests then distilled with excess of alkali. In the first three sets the samples were not taken for the first 30 mins. because the digestion was comparatively slow. The fourth one being very rapid, samples were taken at intervals of 5 mins. The results which have been presented in Figs. 1 and 2 show that (*a*) 'dry' digestion was incomplete even at the end of 3½ hours in one set and 4½ hours in the other ; (*b*) ' Wet ' digestion was speedier than (*a*) and was complete in under 2½ hours ; (*c*) digestion in presence of hydrogen peroxide was still faster and was complete in 1½—2 hours after commencement of boiling ; and (*d*) oxidative digestion was the speediest and was complete in less than 30 mins. in both the sets. These results show conclusively that the last method was the most efficient.

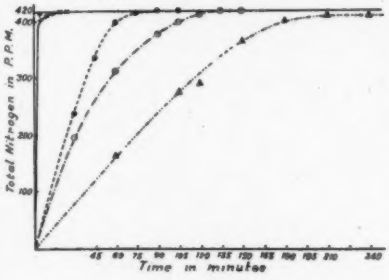

FIG. 1. Rate of Digestion of Black Cotton Soil from Nagpur.

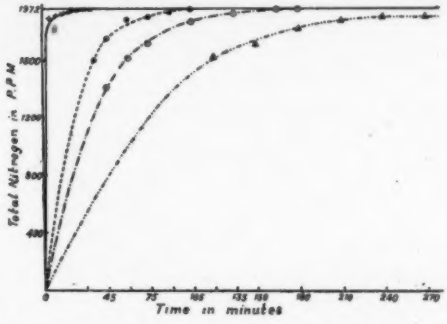

FIG. 2. Rate of Digestion of Alluvial Soil from Travancore.

FIGS. 1 and 2. Comparative Efficiencies of digestion by different methods.

Values obtained by fumeless digestion.
" " wet digestion with H_2O_2.
" " " " H_2O.
" " dry digestion official method.

General Procedure for the Estimation of Nitrogen by Oxidative Digestion.—Based on the foregoing and other observations, the following improved method may be recommended for adoption in routine practice. The material to be digested (10-15 g. in the case of soil ; others in proportion) is weighed out into the distilling flask (Cap. about 1,500 c.c.) and then treated with mercuric oxide (2 g.), water (15 c.c.) and sulphuric acid (10 c.c.). The flask is then fitted with water- or air-cooled condenser and the mixture heated to boiling. After the mixture has started boiling, saturated aqueous solution of chromic anhydride (about 5 c.c.) is introduced through the condenser in small instalments at a time. Boiling is stopped after 30 mins. and the digest, after being cooled for about 5 minutes, diluted with water (about 300 c.c.). It is then treated with sodium sulphite (about 10 g.) and again raised to boil. (The sulphite need not be weighed out but may be added in small instalments at a time until there is a pronounced smell. When the reduction of unused chromic acid is complete, there is also a characteristic change in colour from dark green to blue.) To the boiling mixture, pure zinc (2 g.) is added and the heating continued for a further period of about 5 minutes. The contents of the flask are then cooled and distilled with excess of alkali in the usual way.

Attention has already been drawn to the presence of minute quantities of nitric acid in commercial brands of chromic anhydride and the consequent need for applying some correction. This can be avoided, however, by using pure solid potassium dichromate which can be introduced after momentarily removing the condenser. A very small correction will also be needed for the

nitrogen present in the zinc used for reduction. Commercial samples of dichromate and sulphite may contain some chloride, but the error due to this is entirely eliminated by the presence of the mercury salt in the digesting mixture.

In the hands of the experienced worker, each estimation (commencing from the weighing to the completion of the back titration) will occupy less than 2 hours. If a number of distilling sets are available, at least two dozen determinations can be easily carried out in the course of a working day.

Discussion.

The present enquiry has led to a number of findings of considerable scientific interest. It has thrown fresh light on the mechanism of oxidative digestion; on the nature of the products formed and the manner in which they are retained in the digest. It has also led to the development of an improved method of fumeless digestion which is quite rapid and can be easily applied in routine practice.

The main product of oxidative digestion is ammonia, but a useful proportion is also present in combination with the chromium in the digest. It is difficult to state how exactly the nitrogen is retained by chromium, but there is no doubt that the resulting complex requires treatment with a reducing agent to convert into ammonia. Further work, analytical as well as synthetic, is needed to define the precise nature of the chromium complex and to determine whether one or more of such compounds are present in the digest.

Nitrogen is retained not only by chromium but also by other metals present in the digest. Thus, mercury, copper and bismuth hold a part of the nitrogen and have to be generally treated with a chemical precipitant (usually a sulphide) before the nitrogen can be distilled with alkali in the usual way. In the case of mercury, the nitrogen can also be released by treatment with a reducing agent. The available evidence is not sufficient to show whether nitrogen is retained in the same manner in all the cases.

There are no quantitative data relating to the formation of nitric acid during oxidative digestion. Judging however from the facts that (*a*) only minute quantities of that acid were lost on heating the digest in open flasks and (*b*) the zinc used for reduction cannot deal with more than about 50 p.p.m. of nitric acid, it has to be inferred that the quantities actually formed —at any rate, in the case of soil—are quite small. The mechanism of formation of nitric acid is still obscure.

The most noteworthy feature of oxidative digestion is that no nitrogen is lost through *direct* oxidation. The loss observed in some of the earlier experiments was due to the intermediate formation of ammonium dichromate

which, in turn, was due to the use of potassium dichromate (or chromic acid) as the oxidising agent. Although this difficulty has been overcome by adding the dichromate to the boiling acid mixture, it would nevertheless be desirable to use another oxidising agent, equally efficient in digestion but free from the above defect. Some work in this direction is already in progress.

The advantages of the new method over the others have already been enumerated. Its rapidity and freedom from fumes should commend itself to all workers in the field. The procedure for digestion and reduction are, no doubt, capable of further improvement. The use of the condensers should, if possible, be dispensed with and some simple device introduced for trapping the small quantities of nitric acid that may escape during digestion. The procedure for reduction is rather elaborate and occupies nearly as much time as the digestion itself. It should, if possible, be combined with the distillation. Although the results so far obtained in this direction have not been encouraging, it should still be possible to devise some treatment that can facilitate distillation almost immediately after digestion.

Using dichromate or chromic acid as the oxidising agent, it would be impossible to reduce the proportion of sulphuric acid to water to less than 2 : 1 or, at any rate, below 3 : 2. The reason for this has already been discussed in the text. Although the proposed procedure does not necessitate the use of larger quantities of acid than in the usual Kjeldahl method, it should still be possible to economise further by using other oxidising agents that would function in presence of still more dilute acid. In this manner, the cost of digestion can be reduced and the undue wastage of chemicals (chiefly acid and alkali) avoided.

Among the future lines of interest, mention may be made of the extension of the method to the digestion of organic compounds, particularly those to which the Kjeldahl method is not applicable. The procedure should also be capable of being adapted for micro analysis. Some useful progress has already been made in this direction.

The large amount of information now available in regard to the conditions of digestion should render it possible to include the wet combustion of carbon with the estimation of nitrogen. Some preliminary work in this direction has already shown that using an apparatus of the type described by Subrahmanyan, Narayanayya and Bhagvat (1934), the following conditions should be observed.—(1) for the estimation of carbon, the oxidising agent (chromic acid) should be added to the boiling mixture of the material to be digested with 2 : 1 acid ; (2) the distillation of the vapours (containing CO_2) should be continued for at least 20 minutes ; and (3) after titrating the unused alkali, the distillate (containing all the nitric acid which might have passed over)

should be added to the digest and reduced, in the usual way, with sulphite and zinc prior to distillation with alkali. In this manner, accurate estimates of both carbon and nitrogen in the same samples have been obtained. Attempts are now being made to further simplify the procedure and it is hoped that, before long, it will be possible to develop a comprehensive method that can be easily adopted in routine practice.

Summary.

1. When an organic substance containing nitrogen is digested with a mixture of dichromate (or chromic acid), mercuric oxide, and sulphuric acid (2 : 1), the digest contains the major part of the nitrogen as ammonium sulphate. A small part remains in association with chromium or mercury. Minute quantities of nitric acid are also formed. The estimates of total nitrogen obtained after reducing the digest are lower and less concordant than those obtained by Kjeldahl ' wet ' digestion. This is traced by two causes.—(1) loss of nitrogen in the elementary form when the digestion is conducted according to the above-mentioned procedure and (2) variable nitrogen blank yielded by the large quantities of reducing agent (zinc or reduced iron) used.

2. Comparative study of a number of reducing agents showed that sodium sulphite was the most efficient. When that reagent was combined with small amounts of zinc (for which the nitrogen blank is almost negligible), the highest and the most concordant estimates of total nitrogen were obtained.

Attempts to combine reduction in alkaline medium with distillation of ammonia have not, so far, yielded satisfactory results : nor has combination of other reducing agents (such as oxalic acid or stannous chloride) with zinc or Devarda's alloy proved so efficient as that of sulphite and zinc.

3. The efficiency of digestion was not improved by addition of mineral catalysts. Change in the order of addition of reagents did not lead to any appreciable improvement in the estimate of total nitrogen : nor was any benefit derived by conducting the digestion in two stages.

4. Addition of dichromate (or chromic acid) to the boiling mixture of the material to be digested (soil or other biological material) with sulphuric acid (2 : 1) followed by reduction with sulphite and zinc yielded correct estimates of total nitrogen. There was no need for any extended pre-boiling before addition of the oxidising agent.

5. The small quantities of nitric acid formed during digestion, as also any that may be already present in the original material, are retained in the digest by the use of a condenser. They are reduced by zinc upto a maximum of 50 p.p.m. and included in the estimate of total nitrogen.

6. A study of the mechanism of the loss of nitrogen consequent on adding dichromate (or chromic acid) in the cold showed that (*a*) it is not caused by direct chemical oxidation ; (*b*) the loss of nitrogen occurs between temperatures 130° and 150° which corresponds to the decomposition of ammonium dichromate in a similar medium ; and (*c*) on addition of dichromate (or chromic acid) to the acid mixture at temperatures above 150°, there is no loss of nitrogen. These observations point to the formation of ammonium dichromate at low temperatures followed by its partial decomposition on heating. By adding the oxidising agent to the boiling mixture with acid (temp. 170°) the formation of ammonium dichromate and the attendant loss of nitrogen are avoided. This conclusion is further supported by the facts that (*a*) in the digestion of urea or soil, loss of nitrogen occurs before the acid mixture rises to boil ; (*b*) even after completion of digestion, cooling the digest followed by re-heating causes some loss of nitrogen ; (*c*) on conducting the digestion in presence of 1 : 1 or 1 : 2 acid, the boiling temperature of which is below 160° there is invariably some loss of nitrogen.

7. The conditions relating to oxidative digestion have been standardised and a simple, general procedure developed. (*a*) The use of a condenser is necessary because although, in most cases, very little nitric acid is formed during digestion, there is always the possibility of the original material itself containing some nitrates. A long air-condenser is quite adequate for the purpose. (*b*) A saturated solution of chromic acid can be advantageously used in place of dichromate. (*c*) The digestion proceeds rapidly and accurate estimates of total nitrogen can be obtained only when the proportion of acid to water is at least 2 : 1. (*d*) If the sample is uniform, fineness of division (between particles passing 30 and 90 meshes to the inch) does not make any difference to the accuracy of the estimate of total nitrogen. (*e*) Using soil, nitrogen in quantities upto 30 g. can be easily estimated provided the proportions of acid and oxidising agent can be correspondingly increased.

8. The new method has been successfully applied to the estimation of total nitrogen in soils and biological materials.

9. A study of the comparative efficiency of different methods of digestion showed that the oxidative process was the quickest and the most elegant. Next in order came ' wet ' digestion in presence of hydrogen peroxide and water respectively. ' Dry ' digestion with concentrated sulphuric acid was incomplete even after several hours.

10. The significance of the foregoing and other observations has been discussed. Some fruitful lines of future research have been indicated.

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THE EMBRYOLOGY OF *GISEKIA PHARNACEOIDES* LINN.

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Introduction.

Gisekia is a small genus of African herbs comprising only five species. One of these, *G. pharnaceoides*, which forms the subject of the present paper, extends to India. It is distributed over the greater part of the country, extending from Punjab, Sindh and United Provinces to Ceylon. The plants, however, are not common everywhere, and are mostly confined only to certain kinds of sandy soils.

The genus is of morphological interest on account of its uncertain systematic position. Bentham and Hooker (1862–67) refer it to the family Ficoideæ (Aizoaceæ), where it differs from the rest in possessing an apocarpous gynæcium. Hutchinson (1926) also nearly holds the same opinion and has placed it in his family Molluginaceæ, which he has created by splitting the old family Aizoaceæ into two parts. Baillon (1875) and Engler and Prantl (1889), on the other hand, include *Gisekia* in the Phytolaccaceæ.

The present investigation has been undertaken to see if the embryological study of the genus throws any light on its systematic position. Further the justification of the recent division of the old family Aizoaceæ into Molluginaceæ and Ficoidaceæ by Hutchinson (1926) is also discussed in the light of embryological evidence.

Previous Work.

No work has been done on the embryology of the genus *Gisekia* so far, but the families Phytolaccaceæ and Aizoaceæ as a whole have been studied as follows:

Phytolaccaceæ.—The earlier contributions to the embryology of the Phytolaccaceæ are confined to a single species *Phytolacca decandra*. The earliest one is by Hegelmaier (1885) who has described the development of the endosperm. Next, Lewis (1905) has worked out the whole life-history, though not every part in detail, with the exception of the development of

the embryo-sac. Woodcock (1925) has described the development of the seed, laying special stress on the structure of the endosperm. Besides these investigations, recently Mauritzon (1934) has made a comparative study of the embryology of *Phytolacca octandra*, *Rivina brasiliensis*, *R. humilis*, *Villamilla peruviana* and *Petiveria alliacea*, and a full account of microsporogenesis and structure and development of the embryo-sac of *Rivina humilis* has been given by one of us (Joshi, 1936).

The above studies give the following picture of the embryology of the family :—

Microsporogenesis. The anther wall is 4 cells thick including the epidermis. The innermost of these develops into the tapetum, the next disorganises, and the layer just below the epidermis develops into the fibrous endothecium. The statement of Lewis (1905) that in *Phytolacca decandra* the tapetum is derived from the sporogenous cells is probably incorrect. The tapetal cells are typically 2-nucleate, but according to Lewis (1934) in *Phytolacca decandra* they become ultimately up to 6-nucleate by amitotic divisions. There is no periplasmodium formation. Division of the microsporocytes is simultaneous and takes place through furrowing. The mature pollen has been described only in *Rivina humilis* (Joshi, 1936) and is 3-nucleate at the time of shedding.

Ovule. The ovules are in general campylotropous[1] at the time of fertilisation, except in *Petiveria* where these are amphitropous. There are two integuments, each through its greater length 2 cells thick, and the inner alone takes part in the formation of the micropyle. The cells of the nucellar epidermis divide quite abundantly forming several-layered caps.

Megasporogenesis and Embryo-sac. In general there is a single subepidermal archesporial cell which cuts off a parietal cell. Not seldom, however, there are 2 archesporial cells in *Phytolacca decandra* according to Lewis (1905). In the formation of the megaspores generally no wall is formed between the two nuclei in the upper dyad, so that there are only 3 megaspores of which the upper is 2-nucleate (Mauritzon, 1934). Sometimes even the nuclear division in the upper dyad is suppressed and there are only 3 uni-nucleate megaspores (Joshi, 1936). In exceptional cases the tetrad is T-shaped (Mauritzon).

The chalazal megaspore is always the functional one and develops into an 8-nucleate embryo-sac in the normal manner.

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[1] Mauritzon describes these as amphi-campylotropous.

Endosperm. Endosperm is free nuclear in the early stages. Cell formation starts from the micropylar end. According to Lewis (1905) and Hegelmaier (1885) the endosperm becomes cellular only in the micropylar part, but according to Mauritzon (1934) the endosperm becomes cellular throughout its length. A chalazal accumulation of endosperm is seen just when cell formation starts at its micropylar end. As the seed comes to maturity the endosperm is all digested except a cap-like portion of compressed cells over the micropylar end of the hypocotyl. The storage region of the seed is formed by the perisperm.

Embryo. Embryogeny has not been studied in detail in any species. The first divisions in the fertilised egg are transverse and give rise to a short (*Rivina, Phytolacca*) or long (*Petiveria*) proembryo. Three apical cells of this proembryo give rise to the large part of the embryo proper, as may be judged from the published figures. Suspensor is short in *Rivina* and *Phytolacca* and at least in certain parts is many-seriate. In *Petiveria* the suspensor is very long, narrow and mostly consists of a single row of cells. The mature embryo has the usual curved form characteristic of the Centrospermales.

AIZOACEÆ.—In the family Aizoaceæ, contributions to the embryology of the genus *Mesembryanthemum* have been made by Jönsson (1879), Guignard (1882), D'Hubert (1896), Dahlgren (1916, 1927), Huber (1924), Schmid (1925) and Woodcock (1930). Similarly *Tetragonia* has been investigated by D'Hubert (1896), Dahlgren (1916, 1927) and Huber (1924). Recently Bhargava has described the embryology of *Mollugo nudicaulis* (1934) and *Trianthema monogyna* (1935), including the development of pollen, embryo-sac, endosperm and a few notes on embryogeny.[2] All these investigations may be summarised as follows :

Microsporogenesis. The development and structure of pollen is known only in *Mesembryanthemum pseudotruncatellum* (Schmid, 1925), *Mollugo nudicaulis* (1934) and *Trianthema monogyna* (Bhargava, 1935). It agrees with that of the Phytolaccaceæ except that sometimes (*e.g., Trianthema*) the anther wall including the epidermis is 5-layered, there being formed two layers of cells between the fibrous endothecium and the tapetum.

Ovule. The ovules at the time of fertilisation in *Mesembryanthemum, Tetragonia* and *Mollugo* are ana-campylotropous. In *Trianthema monogyna*

[2] Schnarf (1931) also mentions Hofmeister and Tulasne as contributors to the embryology of the Aizoaceæ, but their work is at present of little more than historical interest.

at the same stage these are nearly campylotropous.[3] There are two integuments in the former genera, but in *Trianthema monogyna* besides these a third integument (or aril) also develops from an early stage and finally surrounds the ovule. The inner integument alone takes part in the formation of the micropyle. In *Mollugo* the cells of the nucellar epidermis lying above and somewhat on the sides of the embryo-sac divide to form a nucellar cap. In *Mesembryanthemum, Tetragonia* and *Trianthema* only the nucellar epidermal cells somewhat on the sides of the embryo-sac divide. Those lying just above the embryo-sac simply stretch out radially.

Megasporogenesis and Embryo-sac. Mostly there is a single hypodermal archesporial cell, but according to Bhargava (1934 and 1935) in *Mollugo nudicaulis* and *Trianthema monogyna* occasionally 2 or 3 megasporocytes are also seen. A parietal cell is cut off. A linear tetrad of megaspores is formed in *Mesembryanthemum floribundum* (Jönsson, 1879), *M. pomederianum* (Dahlgren, 1916), *M. linguiforme* (Huber, 1924) and *Tetragonia expansa* (Dahlgren, 1916 and Huber, 1924). In *Mollugo nudicaulis* there is generally a T-shaped tetrad of megaspores (Bhargava, 1934). In *Mesembryanthemum bulbosum*[4] and *M. Ecklonis* according to Guignard (1882) there are only 3 megaspores. In *Trianthema monogyna* according to Bhargava (1935) there are 3 megaspores of which the micropylar is 2-nucleate, as is commonly the case in the Phytolaccaceæ. The chalazal megaspore in all these cases has been found to be the functional one and develops into the 8-nucleate embryo-sac in the normal manner.

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[3] We have taken considerable care in describing the form of the ovule, as it has been described differently by different authors and in most cases their description does not agree with their figures. In *Mesembryanthemum*, the ovules have been generally described as campylotropous, but the figures of Huber (1924) and Schmid (1925) do not bear out this point. The figures show them really to have a form seen during the development of the campylotropous ovules. This will be at once apparent from a comparison with Fig. 1K of Mauritzon (1934) which shows a stage in the development of the campylotropous ovule in *Rivina brasiliensis*. Both are exactly similar and the form is nearly intermediate between that of anatropous and campylotropous ovules. The term ana-campylotropous has, therefore, been used in describing such ovules in the present paper.

The form of the ovules in *Mollugo nudicaulis* as seen from the figure of Bhargava (1934, Fig. 1) is similar to those of *Mesembryanthemum*, but in describing them the author makes two contradictory statements. In summarizing the previous work on the embryology of the Aizoaceæ, he states that the ovules of *Mesembryanthemum* are campylotropous, while he describes the ovules of *Mollugo nudicaulis*, which have very nearly the same form, as anatropous.

The ovules of *Trianthema monogyna* are described by Bhargava (1935, p. 51) as amphitropous, but from his figure they appear to be more like campylotropous and should be described as such or as amphi-campylotropous.

[4] Bhargava (1934) has wrongly referred to *M. corymbosum*.

In *Mesembryanthemum pseudotruncatellum* according to Schmid (1925) the megaspore-mother cell directly develops into the embryo-sac and its development corresponds to the "Lilium-type", but these observations need confirmation before they can be accepted, as such a development has not been seen in any other member of the family.

The mature embryo-sac in *Mesembryanthemum, Tetragonia, Aizoon* and *Trianthema* contains abundant starch grains, but these are absent from the mature embryo-sac of *Mollugo nudicaulis.*

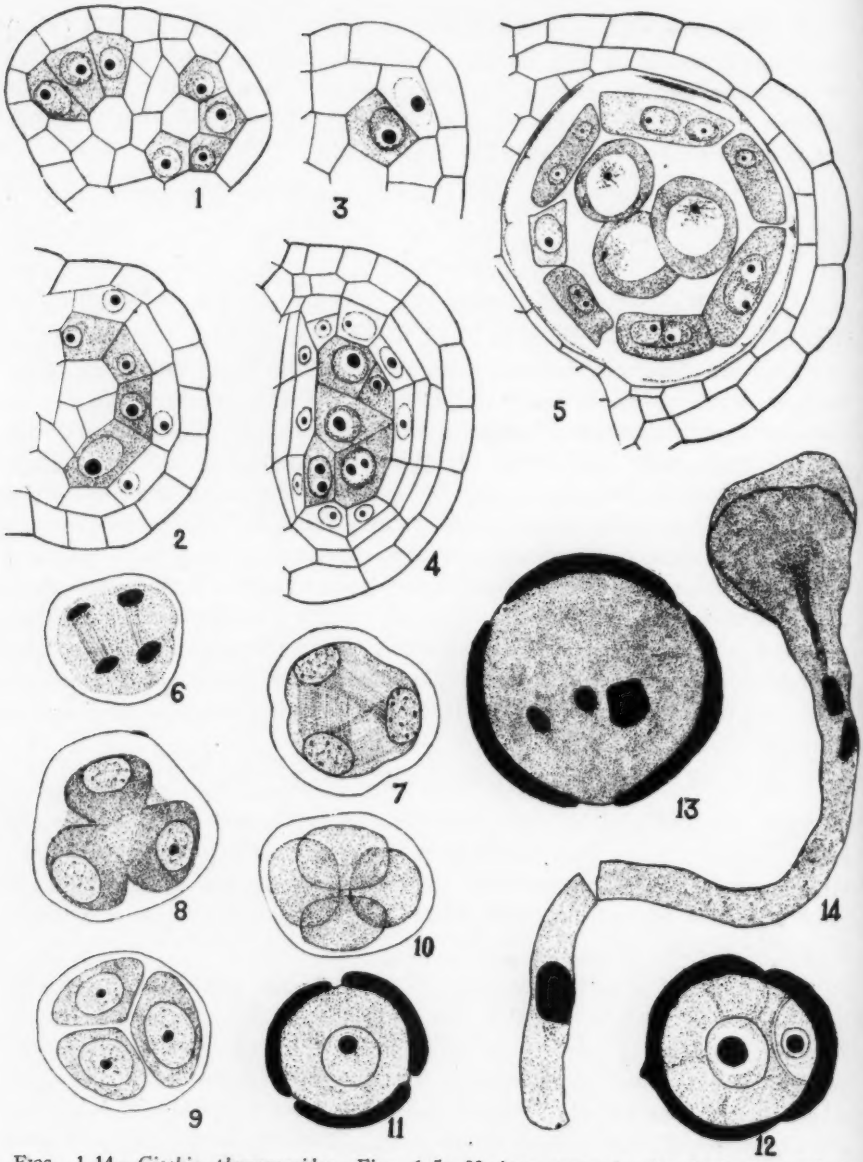
Endosperm. The development and structure of the endosperm and perisperm agrees with that of the Phytolaccaceæ.

Embryo. There is no good account of embryo development available for any member of the Aizoaceæ. Only a few scattered observations can be collected from the works of Dahlgren (1916), Huber (1924), Woodcock (1930) and Bhargava (1934 and 1935). It appears from the last author's figures that a proembryo of 6 or more cells is formed by transverse divisions in the oospore before longitudinal divisions set in. The 3 or 4 apical cells of this give rise to the embryo proper. The suspensor is uniseriate in *Mollugo nudicaulis* (Bhargava, 1934), mostly uniseriate and with a large basal cell in *Mesembryanthemum linguiforme* (Huber, 1924), uni-few-seriate in *M. crystallinum* (Woodcock, 1930), several-seriate and massive in several species of *Mesembryanthemum, Tetragonia expansa* (Dahlgren, 1916) and *Trianthema monogyna* (Bhargava, 1935). The mature embryo is curved as usual in the Centrospermales.

Material and Methods.

The material used in the present investigation was collected by the senior author during July and August 1931 from near Hoshiarpur, a sub-Himalayan locality in the Punjab. The plants of *Gisekia pharnaceoides* are quite abundant here on the sandy banks of a broad seasonal stream which is locally known as *Cho.*

The material of early stages was fixed in chromo-acetic solution and of later stages in Allen's modified Bouin's fluid and formalin-acetic-alcohol. It was embedded in paraffin according to the customary methods. The younger flowers were microtomed at a thickness of 6–8 μ, while the older stages were cut at 10–12 μ. Safranin and G. Violet and Heidenhain's iron-alum-hæmatoxylin were used for staining. With the latter stain, Tuan's method of destaining with picric acid was followed, and better results were obtained than by destaining with iron-alum.



FIGS. 1–14.—*Gisekia pharnaceoides*. Figs. 1–5.—Various stages in the development of an anther-lobe; Figs. 1, 2 and 4, longitudinal sections; Figs. 3 and 5, transverse sections. Fig. 6.—A microsporocyte in homotypic metaphase showing a parallel arrangement of spindles. Fig. 7.—A microsporocyte after the homotypic division

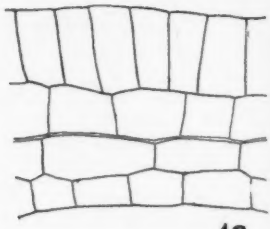
showing grand-daughter nuclei arranged in a tetrahedral manner and secondary spindle fibres. Fig. 8.—A microsporocyte showing cytokinesis by furrowing. Figs. 9 and 10.—Tetrahedral and bilateral pollen tetrads inside the mother-cell wall. Figs. 11, 12 and 13.—1-nucleate, 2-nucleate and 3-nucleate pollen grains respectively. Fig. 14.—A germinating pollen grain found near the base of the gynæcium. Figs. 1–5 × 640; Figs. 6–14 × 1260.

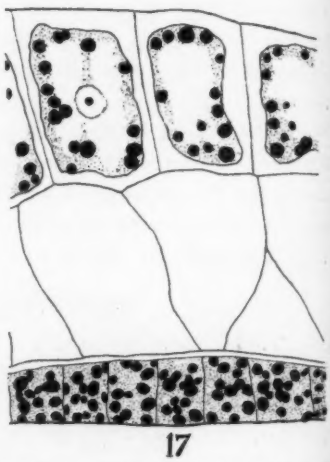
Organogeny of the Flower.

The development of the flower of *Gisekia pharnaceoides* shows a general resemblance with that of *Phytolacca decandra* (Lewis, 1905). The flower rudiment first appears as a rounded structure. From its sides appear the various floral parts in strict acropetal succession, the 5 tepals appearing first, followed by the 5 stamens and last of all the 5 carpels. The carpel rudiments are at first boat-shaped and arch over the growing point of the flower (Fig. 19), from the border of which appear the ovules, one in the axil of each carpel. Further development of each carpel is very similar to that of *Ranunculus*, as described by Salisbury (1931). More cases of similar carpel development have been enumerated recently by Thomas (1934), and its significance has been discussed by one of us elsewhere (Joshi, 1936).

Development of Pollen and Male Gametophyte.

The stamen carrying a 4-lobed anther develops in the normal manner. The primary archesporium as it differentiates in each anther-lobe consists of a single hypodermal row of 3 or 4 cells (Figs. 1 and 2). These cells divide by periclinal walls giving rise to a layer of primary parietal cells towards the outside and a layer of sporogenous cells towards the inside (Figs. 2 and 3). The primary parietal cells further divide by periclinal walls to give rise to two layers of cells. The outer one of these does not divide further, but the cells of the inner one undergo one more periclinal division. Thus besides the epidermis, three layers of parietal cells are formed to the outside of the sporogenous tissue (Fig. 4). The cells of the innermost of these layers increase in size, become densely filled with cytoplasm, begin to stain deeply and thus develop into the tapetum. At first they are uni-nucleate like other cells, but from the time of synizesis in the sporocytes they become 2-nucleate (Fig. 5) by a mitotic division of their nucleus. The sub-epidermal layer of parietal cells develops into the fibrous endothecium. The layer of parietal cells in between the tapetal and the endothecial layers is crushed early due to the growth of the two latter (Fig. 5). The structure of the anther wall of *Gisekia pharnaceoides* is therefore quite similar to that of *Rivina* (Joshi, 1936), *Mollugo* (Bhargava, 1934) and *Mesembryanthemum* (Schmid, 1925). In *Trianthema monogyna* (Bhargava, 1935), as stated previously, there are 4 layers of parietal tissue in the anther.





FIGS. 15–17.—*Gisekia pharnaceoides*. Fig. 15.—Longitudinal section of an ovule inside the carpel at the time of fertilization. A pollen-tube is seen entering the embryo-sac. Figs. 16 and 17.—Portions of testa showing the structure of the integuments; Fig. 16, from an ovule containing a 1-nucleate embryo-sac; Fig. 17, from an ovule showing a mature embryo-sac. Fig. 15 × about 180; Figs. 16 and 17 × 800.

The primary sporogenous cells divide about three times and give rise to about 24–32 microspore-mother cells in each lobe of the anther. The output of pollen from each stamen varies approximately from 400–480.

The two meiotic divisions are quite normal. During the homotypic division the spindles are generally arranged at right angles to each other, but rarely they are also found to show a parallel arrangement (Fig. 6). The four nuclei formed in the pollen-mother cells as a result of the two meiotic divisions become connected by secondary spindle fibres (Fig. 7). Cytokinesis takes place by simultaneous furrowing from four sides (Fig. 8). The young pollen grains are mostly arranged in a tetrahedral manner (Fig. 9), but bilateral arrangement is also seen in a few cases (Fig. 10).

Soon after liberation from the mother-cell wall the pollen grains round off. They develop the usual exine and intine. The former is perforated by 3 germ pores, just as in *Mesembryanthemum*, *Mollugo* and *Trianthema* (Fig. 11). The nucleus divides giving rise to a large tube nucleus and a smaller generative nucleus. The tube nucleus has one or two nucleoli. The generative nucleus is at first cut off from the tube nucleus by a curved wall forming a lenticular generative cell (Fig. 12). Later on this wall disappears and the generative nucleus divides giving rise to two male nuclei (Fig. 13), just as in *Mollugo nudicaulis* (Bhargava, 1934). There is no organisation of male cells as has been reported in *Rivina humilis* (Joshi, 1936) and *Trianthema monogyna* (Bhargava, 1934). The pollen grains are shed in this 3-nucleate condition (Fig. 13) and measure at this time about 30 μ in diameter.

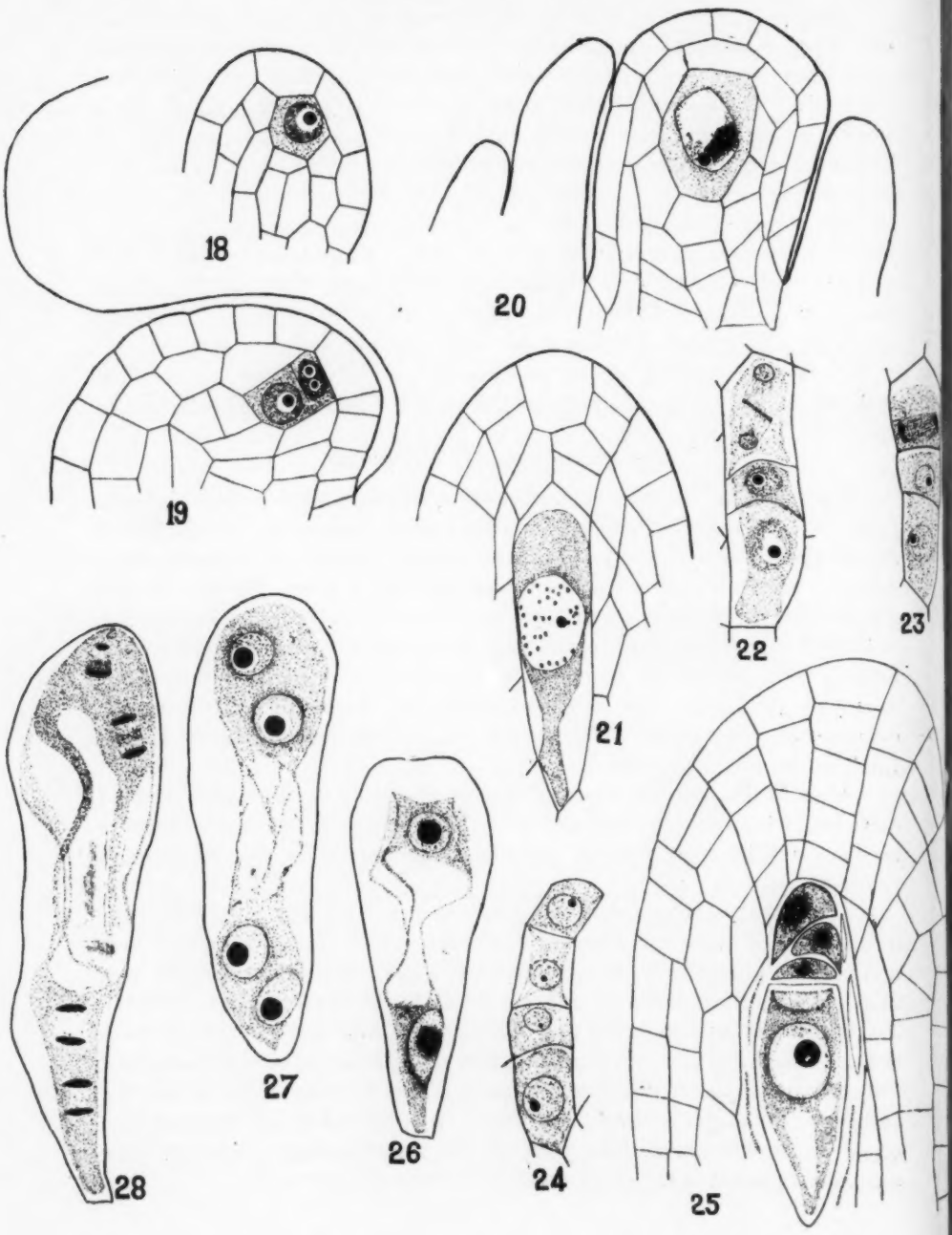
During germination of the pollen grains, the tube nucleus comes out first and in the pollen-tube remains ahead of the two male nuclei (Figs. 14 and 34).

Megasporogenesis and Embryo-sac.

The ovule at the time of fertilisation is ana-campylotropous in form (Fig. 15), as in *Mollugo nudicaulis* (Bhargava, 1934) and in species of *Mesembryanthemum* (Huber, 1924; and Schmid, 1925). It measures about 500 μ in length and shows a short funiculus and a short raphe. The two integuments are both through their greater part two cells thick (Fig. 16), but in the region of the micropyle they may be up to 3 or 4 cells thick. The inner projects beyond the outer and alone takes part in the formation of the micropyle (Fig. 15). The nucellus consists of 4 layers of cells above the embryo-sac, 3 or 4 layers on the sides of the embryo-sac and is about 10 cells thick below the embryo-sac.

After fertilisation the chalazal end of the ovule bends slightly further downwards towards the funiculus and the micropyle. The ovule therefore becomes a little more inclined towards campylotropy in the seed stages (Figs. 51 and 52).

The primary archesporium differentiates at a very early stage during the development of the ovule, even before the integument initials have appeared, and consists always only of a single cell (Fig. 18), though occasionally 2 or more sporogenous cells have been found in *Mollugo* (Bhargava, 1934), *Trianthema* (Bhargava, 1935) and *Phytolacca* (Lewis, 1905). The division of the archesporial cell by a periclinal wall to form the primary wall cell and the megaspore-mother cell, the beginning of the bending of the ovule, the arching of the carpel rudiment over the apex of the flower and the initiation of the inner integument take place almost simultaneously (Fig. 19). The second integument of the ovule develops a little later.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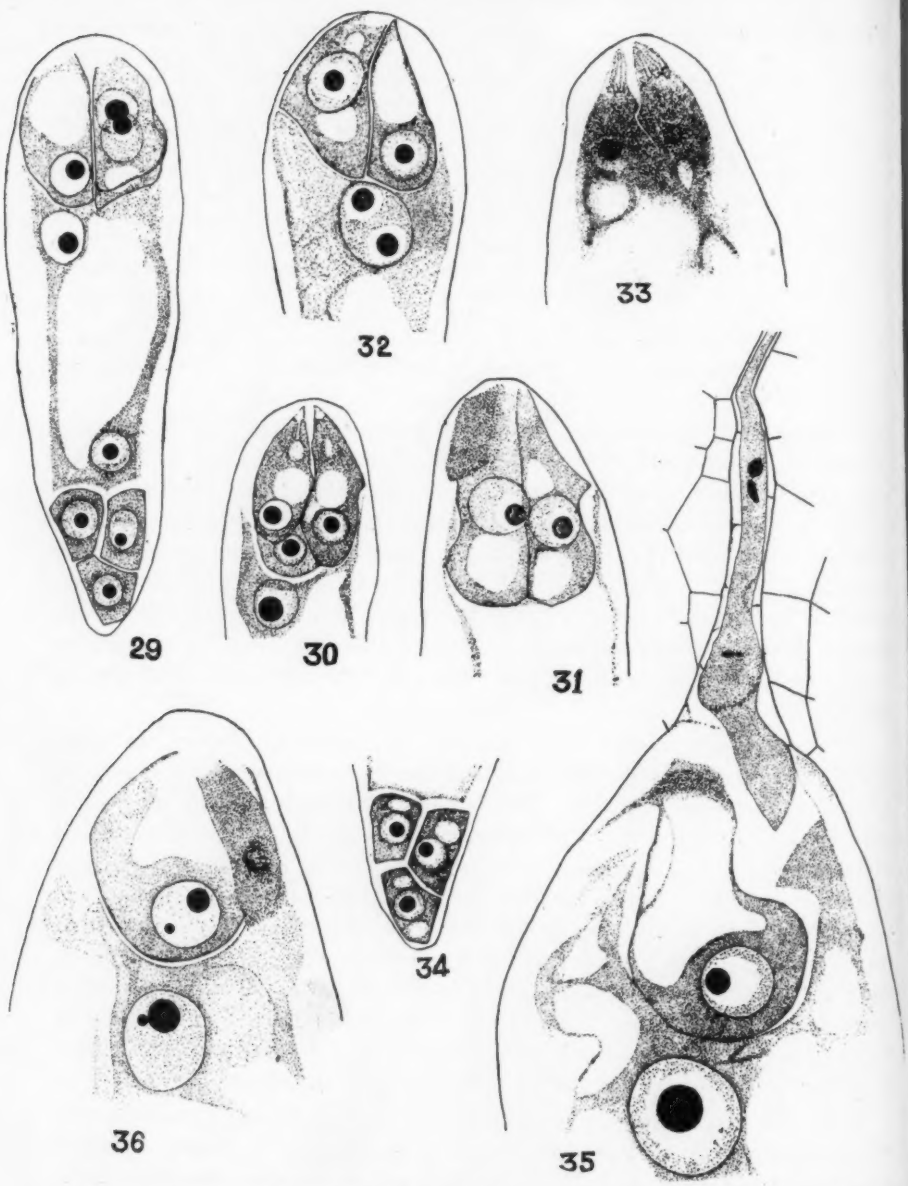
FIGS. 18–28.—*Gisekia pharnaceoides.* Various stages in megasporogenesis and development of the embryo-sac. Fig. 18.—A young ovule showing primary archesporium. Fig. 19.—An ovule with the carpel rudiment arching over it and showing the differentiation of the primary wall cell and the megaspore-mother cell. Fig. 20.—Apical portion of an ovule with the integuments and the megaspore-mother cell in synizesis. Fig. 21.—Apical portion of nucellus showing divisions in the epidermal cells and megaspore-mother cell in diakinesis. Fig. 22.—A stage in the development of megaspores; the lower dyad has completed its division and given rise to two megaspores, the upper is in telophase. Fig. 23.—Same as Fig. 22, but the spindle in the upper dyad is transversely orientated. Fig. 24.—A linear tetrad of megaspores. Fig. 25.—Apical portion of the nucellus of an ovule showing the development of parietal tissue, the epidermal cap, and four megaspores of which the chalazal is enlarging and the other three are degenerating. Fig. 26.—A 2-nucleate embryo-sac. Fig. 27.—A 4-nucleate embryo-sac. Fig. 28.—A stage in the development of the 8-nucleate embryo-sac showing four nuclei in telophase. × 800.

The primary wall cell does not undergo many divisions. It divides once or more anticlinally and its daughter cells undergo one periclinal division and thus give rise to 2 layers of parietal tissue (Figs. 21 and 25). The epidermis of the nucellus above the embryo-sac divides and gives to 2-3 layers of cells,—3 layers just above the embryo-sac and 2 layers towards its upper corners (Figs. 21 and 25). In this manner 5 layers of cells are formed above the uni-nucleate embryo-sac (Fig. 21). The innermost of these is crushed by the further growth of the embryo-sac. In the fertilisable ovule for this reason there are 4 layers of nucellar cells above the embryo-sac, as mentioned previously.

The megaspore-mother cell enters upon its first division when the ovule has become nearly completely bent, and the integuments have reached the level of the nucellus (Fig. 20). The anthers of the flower at this stage show uni-nucleate pollen grains. This division is quite normal. During the second division, the chalazal dyad always divides before the micropylar. By the time the latter begins to divide, the division is complete in the lower (Figs. 22 and 23). The spindle in the micropylar dyad is generally arranged in an oblique manner (Fig. 22), but in one instance it was seen in a transverse plane (Fig. 23). Such a condition would have naturally given rise to a T-shaped tetrad of megaspores. In all other instances the megaspores have been seen to be arranged in a linear manner (Fig. 24). The condition of the megaspores in other investigated Phytolaccaceæ and Aizoaceæ has been mentioned previously.

The chalazal megaspore enlarges and functions as the embryo-sac-mother cell, while the other three degenerate (Fig. 25). It developes into the 8-nucleate embryo-sac through the usual 2- and 4- and 8-free-nucleate stages (Figs. 26–28). The egg-apparatus, the polar nuclei and the antipodals are organised in the normal manner (Fig. 29).



FIGS. 29–36.—*Giseckia pharnaceoides*. Fig. 29.—A young fully-formed embryo-sac showing an egg-cell, two synergids with normal chalazal vacuoles lying over each other, two polar nuclei and three antipodals. Fig. 30.—Micropylar part of an embryo-sac

showing the egg-apparatus and the micropylar polar nucleus. The synergids show vacuoles above their nuclei and pointed lightly staining apices. Below the apex in each synergid there is another small vacuole opening at the apex by a narrow canal. Fig. 31.—Micropylar portion of an embryo-sac showing two synergids, one with its vacuole below the nucleus and the other with two vacuoles in place of one, one below and the other above the nucleus. Fig. 32.—Micropylar portion of an embryo-sac showing one egg, one synergid and two polar nuclei fusing with each other. Fig. 33.—Micropylar portion of an embryo-sac showing two degenerating synergids with 'filiform apparatus' at their apices. Fig. 34.—Chalazal end of an embryo-sac showing three antipodal cells with vacuoles. Fig. 35.—Micropylar portion of nucellus and embryo-sac at the time of fertilization, showing the egg, the secondary nucleus and a pollen-tube with two male nuclei and one (not properly cut) tube nucleus entering the embryo-sac. Fig. 36.—Double fertilization. One male nucleus has fused with the nucleus of the egg, the other with the secondary nucleus, and the tube nucleus is seen degenerating inside the apex of the pollen-tube. × 800.

The egg-cell as usual is a broadly flask-shaped structure with a large vacuole towards its micropylar end and the nucleus embedded in cytoplasm pressed against the chalazal end (Figs. 29, 35 and 36). The synergids are of nearly the same size as the egg-cell or in early stages may be even slightly bigger. The vacuolation in the synergids has been found to be very variable. In the majority of cases observed (about 70%), the large vacuole in both the synergids occurs below the nucleus (Fig. 23), as is the general condition in the angiosperms and has also been found to be the rule in other Phytolaccaceæ and Aizoaceæ. In other embryo-sacs both the synergids have been found to show their vacuole in the upper part, *i.e.*, above the nucleus, just like the egg-cell (Fig. 30). The intermediate condition is also seen where one synergid of an embryo-sac has a vacuole towards the upper and the other towards the lower side of the nucleus. In other instances synergids are seen with 2 vacuoles, one above and one below the nucleus (Figs. 31 and 32). Similar variation in vacuolation and the formation of egg-like synergids has been recorded in very few instances among the very large number of flowering plants investigated so far. Osterwalder (1898) has noted this condition in *Aconitum Napellus*, Persidsky (1914) in *Delphinium elatum* and Frisendahl (1912) in *Myricaria germanica*. Recently Puri (1934) recorded in *Moringa oleifera* certain cases of embryo-sacs in which one or both the synergids assume the form of the egg due to the migration of the nucleus towards the lower side, and similar egg-like synergids have also been seen by Joshi and Kajale (unpublished paper) in *Tamarix dioica*. In all these cases though the synergids present the appearance of an egg, in no case they have been seen to be fertilised. The synergids of *Gisekia pharnaceoides* generally show small indentations in their upper part (Figs. 30 and 31, right hand synergid). They have short acute apices which do not stain heavily with hæmatoxylin. Below the apex is often

visible a small vacuole besides the large vacuole of the synergid. This seems to open at the apex by a narrow canal (Fig. 30). It is this vacuole which probably secretes the substance which guides the pollen-tube to the embryo-sac and the narrow canal is the channel through which it flows out. As the synergids begin to degenerate, longitudinal striations appear in their apices giving rise to a ' filiform apparatus ' (Fig. 33). The three antipodals are arranged in a triangular fashion, one in the chalazal end of the embryo-sac, the other two on the micropylar face of the first (Figs. 29 and 34). In the mature embryo-sac they generally develop one or two quite distinct vacuoles (Fig. 34) and persist up to the time of fertilisation (Fig. 15). In *Mollugo nudicaulis* according to Bhargava (1934) the antipodals are ephemeral and degenerate even before the fusion of polar nuclei, but in *Phytolacca decandra* (Lewis,1905) and *Rivina humilis* (Joshi, 1936), the antipodals persist up to fertilisation as reported above for *Gisekia pharnaceoides*. The polar nuclei are at first situated at the opposite ends of the embryo-sac (Fig. 29). Afterwards the chalazal moves upwards and both take up a position below the egg-apparatus. Here they fuse (Fig. 32) and give rise to a large secondary nucleus (Fig. 35).

The mature embryo-sac measures about 160 μ in length. It is broad towards the micropylar end and narrowed towards the chalazal end (Fig. 15). It agrees with the embryo-sac of *Mollugo nudicaulis* (Bhargava, 1934) in showing absence of starch grains.

Fertilisation.

Fertilisation is porogamous (Figs. 15 and 25). By the time the pollen-tube has traversed the micropyle and enters the embryo-sac, the synergids degenerate (Fig. 35). The egg has greatly increased in size and is bulged out at its lower end. The secondary nucleus lies not far away from the egg. The pollen-tube brings with it two more or less oblong male nuclei and a tube nucleus, and passes down along one side of the egg. The tip of the pollen-tube becomes somewhat swollen inside the embryo-sac. The male nuclei inside the pollen-tube remain at first far behind the tube nucleus (Figs. 14 and 35), but about the time of fertilisation come ahead and are discharged first. The tube nucleus seems to remain behind in the tip of the pollen-tube and degenerates there (Fig. 36). The oblong male nuclei on coming in contact with the egg and the secondary nuclei change their shape and become spherical. Normal double fertilisation takes place (Fig. 36).

Endosperm.

After fertilisation, the development of the endosperm starts before the first division in the oospore. The embryo-sac shows several endosperm nuclei when the oospore is dividing or there is a 2-celled proembryo. The

development of the endosperm agrees with that of other investigated Phytolaccaceæ and Aizoaceæ. At first it develops in a free nuclear manner. Cell formation begins at the micropylar end of the embryo-sac as the cotyledons begin to differentiate in the embryo (Fig. 51), and progresses towards the chalazal end. During the development of the endosperm (and also the embryo) the embryo-sac becomes greatly elongated and assumes the curved form characteristic of the Centrospermales. In the mature seed the entire endosperm is used up by the growth of the embryo, except at the micropylar end, where it persists as a small cap of nearly crushed dark-staining cells, a little more extensive towards the outside of the embryo than the inside (Fig. 52).

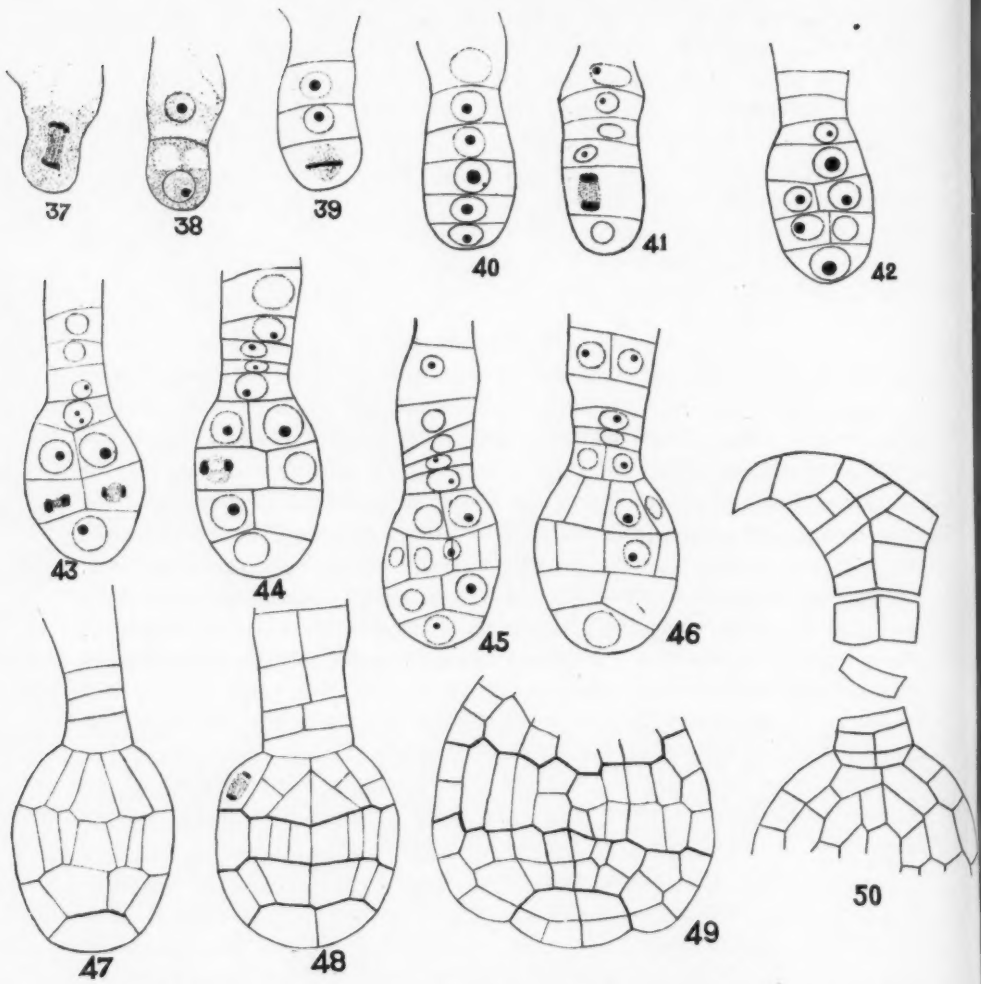
Embryogeny.

Proembryo. The first division in the fertilised egg in *Gisekia pharnaceoides* is transverse (Fig. 37) and gives rise to two cells (Fig. 38). By further transverse divisions a proembryo of 7 or 8 cells is formed before any longitudinal walls appear (Figs. 39–41).

Development of the embryo proper. Four apical cells of the proembryo take part in the formation of the embryo proper excluding the dermatogen of the root apex and the root cap. The rest of them give rise to the suspensor, except that a daughter cell of one of them adjacent to the embryonic cells (hypophysis) gives rise to the dermatogen of the root apex and the root cap. The cells of the proembryo taking part in the formation of the embryo proper may be numbered from the apex of the proembryo backwards as I, II, III and IV. These cells or the tiers to which they give rise do not develop simultaneously during the differentiation of the embryo. The I (apical) lags behind all others in its development. The II and III are the first to divide and are followed by divisions in the IV (Figs. 39 and 40). The ultimate fate and development of the different embryonic initials is as follows :–

I. As mentioned above, this cell is very slow to divide. It divides first by two longitudinal walls at right angles to each other, when the differentiation of three histogenic layers has already taken place in the other three tiers (Fig. 48). Next its daughter cells divide periclinally, when the cotyledons are just being initiated and the hypophysis has divided longitudinally (Fig. 49). By further divisions the stem tip is formed from this tier of cells.

II. The cell just behind the apical cell of the proembryo gives rise to the cotyledons and the portion of the axis in between the cotyledon-bases. This is the first cell to enter into divisions along with the III (Fig. 42), but after the first two divisions, which result in the formation of quadrants, its rate of division slows down. The first division in its quadrants is periclinal and separates the dermatogen (Fig. 47). The next division in the inner



FIGS. 37–50.—*Gisekia pharnaceoides*. Various stages in the development of the embryo. Figs. 49 and 50 are parts of two embryos at nearly the same stage of development. Fig. 49 shows the apical portion and Fig. 50 the suspensor and the hypophysis. × 460.

daughter cells is again periclinal (Fig. 48). By this time the development of III and IV tiers has progressed much further. Exceptionally the first periclinal divisions in the quadrants of the II tier take place before those of the III and IV tiers (Fig. 43). After the two periclinal divisions in the quadrants of the II tier a number of divisions take place on either side in all

planes forming two cell masses, each of which gives rise to a cotyledon (Fig. 49). The inner cells of this tier differentiate into the periblem and plerome of the embryonic axis.

III and IV. These two cells give rise to the hypocotyl and periblem and plerome of the root.

The divisions in the III take place a bit earlier than in the IV. After the formation of the quadrants (Figs. 42–44) by periclinal divisions the dermatogen is differentiated (Figs. 45 and 46). The inner region is next marked out into periblem and plerome by the following periclinal divisions.

In the IV tier also the quadrants are differentiated first by two longitudinal divisions at right angles to each other. These are followed by obliquely periclinal divisions in the 4 quadrants resulting in the differentiation of the dermatogen (Fig. 46). The next periclinal divisions in the inner cells differentiate the periblem and the plerome (Figs. 47 and 48).

After the differentiation of the three histogenic layers in all the tiers, cells of the dermatogen divide only by anticlinal walls, while those of periblem and plerome divide in all planes (Figs. 48 and 49).

Differentiation of the root-tip. The cell of the suspensor next to the IV embryonic cell divides transversely. Its daughter cell adjacent to the embryonic mass is the hypophysis. By its longitudinal (Fig. 46) and transverse (Fig. 50) divisions, it gives rise to the dermatogen of the root-tip and the root-cap.

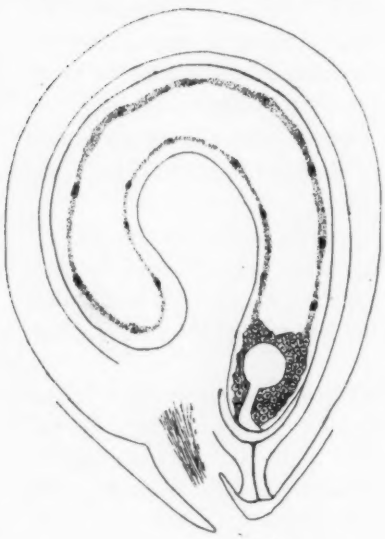
The development of the embryo of *Gisekia pharnaceoides* thus agrees with that of *Sagina procumbens* as described by Souèges (1924), except that in the latter plant only three cells take part in embryo-formation excluding the dermatogen at the apex of the radicle and the root-cap and there is only a single cell in place of the III and IV cells of *Gisekia*.

Suspensor. In the proembryo there are 4 suspensor cells. These undergo transverse divisions so that the suspensor becomes 9–10 cells long. It remains uni-seriate close to the embryonic mass, but in its basal portion longitudinal divisions appear. Here it becomes 2–3 cells broad and assumes a characteristic beak-like form (Fig. 50).

Mature embryo. Growth on one side of the embryo is generally greater than on the other, so that it bends and follows the curved form of the embryo-sac (Fig. 52). It completely absorbs the endosperm except a small portion forming a cap over the root which has already been described, but a large part of the nucellus enclosed in the curve of the embryo is left behind in the mature seed forming the perisperm. Both the embryo and the perisperm are loaded with starch grains. No starch is stored in the endosperm.

Differentiation of the Testa.

As has been described before, both the integuments of the ovule are through their greater part two cells thick (Fig. 16). They remain so up to the 4-nucleate or early 8-nucleate embryo-sac stage. By the time of fertilisation, the outer layer of the inner integument is crushed by the growth of the two layers of the outer integument. By the same time large granules appear in the cells of the outer layer of the outer integument and the inner layer of the inner integument (Fig. 18), as has been noted in several other Centrospermales. In the mature seed these two layers of cells become greatly thickened and hardened.



51

52

FIGS. 51–52.—*Giseckia pharnaceoides*. Fig. 51.–Longitudinal section of an ovule showing an embryo (represented in white) at the stage sketched in Figs. 49 and 50, and endosperm cellular in the micropylar part of the embryo-sac and free nuclear in the rest of the embryo-sac. Fig. 52.—Longitudinal section of an achene containing a mature seed inside. The pericarp in the figure is striated, testa is shown in white, the embryo is deeply shaded, the crushed cells of the endosperm cap over the radicle are made in black and the starchy perisperm is lightly shaded. Fig. 51 ×80; Fig. 52 ×60.

Discussion.

On account of their close relationship, the embryological features of the two families Phytolaccaceæ and Aizoaceæ in many respects are so similar, that it becomes difficult to decide definitely the group in which the genus

Gisekia should be placed. The process of microsporogenesis (including the origin of the tapetum and the form of the mature pollen), the development of the embryo-sac, the development and structure of the endosperm, and the structure of the mature seed are quite similar in both. The number, structure and arrangement of the megaspores is so variable even in the same family or even in the same species, that it does not give any help in solving the question. The development of the embryo is not known in detail in any of the previously investigated plants, both of the Phytolaccaceæ and the Aizoaceæ. Therefore one has only to depend on the form of the ovule, the structure of the nucellus and the structure of the mature embryo-sac in deciding the relationships in these families on an embryological basis.

Comparison of these characters shows that the genera *Mesembryanthemum*, *Trianthema* and *Teragonia* stand quite apart from the rest. In them the cells of the nucellar epidermis just above the embryo-sac do not divide, but simply stretch out radially, and the mature embryo-sac is full of starch grains. As these characters appear to be quite constant, it seems necessary that these genera should be separated from the rest. The embryological studies therefore support the views of Hutchinson (1926) who has placed Ficoidaceæ (including the above genera) as a separate family from the Molluginaceæ. The anatomy of the vegetative parts of these plants also supports such a conclusion. Most genera of the Ficoidaceæ are known to show anomalous secondary growth by a succession of secondary cambiums in their root and stem (Solereder, 1908). In the Molluginaceæ, on the other hand, this type of secondary thickening in the axis is very rare and is known only in one or two forms.

The Phytolaccaceæ and the Molluginaceæ have got very similar structure, but they differ in the form of the ovule and the size of the nucellar epidermal caps. The fertilisable ovule in the Phytolaccaceæ in general is campylotropous or amphi-campylotropous, rarely amphitropous, and the nucellar epidermal caps are generally very thick. In *Mollugo* the ovule is anacampylotropous as in *Mesembryanthemum* and the nucellar epidermal caps are generally only a few-layered. The genus *Gisekia* in these respects agrees with *Mollugo* and *Mesembryanthemum*, and therefore should be placed in the family Molluginaceæ.

Summary.

1. The primary archesporium in each of the anther-lobes consists of a hypodermal row of 3 or 4 cells. The primary wall cells give rise to 3 layers of cells, the innermost of which develops into bi-nucleate tapetal cells, the middle is crushed by the growth of the other layers, and the outermost

develops into the fibrous endothecium. Cytokinesis takes place by furrowing. Young pollen grains are mostly arranged in a tetrahedral manner, rarely bilaterally. The pollen output per anther is about 400–480. The mature pollen-grains are 3-nucleate and show generally 3 germ pores. During the growth of the pollen-tube, the tube nucleus for the most part remains ahead of the male nuclei.

2. The ovule is ana-campylotropous. The nucellar epidermis above the embryo-sac gives rise to a cap of 2–3 layers. There is a single primary archesporial cell which cuts off a wall cell. The wall cell gives rise to two layers of parietal tissue. The megaspore-mother cell gives rise generally to a linear, rarely T-shaped, tetrad of megaspores. The chalazal megaspore always develops into the embryo-sac.

3. Development of the embryo-sac is normal. The synergids develop small indentations in their upper part. Very often they show vacuolation of the egg type. Their pointed apices do not stain deeply with hæmatoxylin. A little behind the apex a small vacuole is often seen besides the large vacuole of the synergids. This seems to open by a small canal at the apex of the synergids and probably secretes the substance which guides the growth of the pollen-tube towards the embryo-sac. About the time of degeneration, the apices of the synergids show striations and present the appearance of a 'filiform apparatus'. The egg has got the normal structure. The antipodals develop small vacuoles. The polar nuclei fuse below the egg-apparatus to give rise to a large secondary nucleus. The mature embryo-sac does not show any starch grains.

4. Fertilisation is porogamous. Double fertilisation has been observed.

5. Development and structure of the endosperm and the structure of the mature seed agrees with that of other investigated Phytolaccaceæ and Aizoaceæ (Molluginaceæ and Ficoidaceæ).

6. In the development of the embryo, the oospore by transverse divisions gives rise to a uni-seriate proembryo of about 8 cells. Four terminal cells of this proembryo take part in the development of the embryo proper. The apical one gives rise to the stem tip, the sub-apical one to the cotyledons and the portion of the axis in between them, and the third and fourth cells from the apex give rise to the hypocotyl and periblem and plerome of the root. The dermatogen of the root-tip and the root-cap are formed from a daughter cell of one of the adjacent suspensor cells (the hypophysis). The suspensor by transverse divisions becomes 9–10 cells long and by longitudinal divisions in its basal part 2–3 cells broad.

7. From a comparison of the embryological features of Phytolaccaceæ, Aizoaceæ and *Gisekia*, it is concluded that the family Aizoaceæ (Ficoideæ of Bentham and Hooker) be separated into two families Molluginaceæ and Ficoidaceæ, as Hutchinson has done, and the genus *Gisekia* should be placed in the Molluginaceæ.

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THE MYXOPHYCEAE OF THE BOMBAY PRESIDENCY, INDIA.—I.

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Received December 26, 1935.
(Communicated by Prof. Y. Bharadwaja, M.Sc., Ph.D. (Lond), F.L.S.)

Hansgirg was one of those persons who travelled a lot in tropical countries and, though principally engaged in other ways, made large collections of plants. He collected some Myxophyceæ from the Bombay Presidency, and these algæ were later determined and recorded by others. After Hansgirg no record was made of the blue-green algæ of this province. Professor V. N. Hate, however, started collecting these algæ as late as 1922, and he was followed by the writer in the same pursuit in 1927. It is now desired to record the whole myxophyceous flora of this Presidency in parts, and the present account relates to the Myxophyceæ of the Bombay Islands[1] only.

Hansgirg's collections, as recorded by De Toni,[2] reveal that he established only nine new species from Bombay proper. The writer, in spite of careful search, has not been able to come across five of them, *viz.*, (1) ***Merismopedium minimum***[3] G. Beck, (2) ***Phormidium Hansgirgi*** Schmidle, (3) ***Campylonema indicum***[4] Schmidle, (4) ***Hapalosiphon Hansgirgi***[5] Schmidle, and (5) ***Leptochæte Hansgirgi*** Schmidle.

Bombay has no natural reservoirs, but there are a few mill and temple tanks. The Salsette island, however, has good many brooks, rivulets and

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[1] The Bombay Islands (*viz.*, Bombay proper and Salsette) are situated in latitude 18° 54′ N. and longitude 72° 49′ E., being almost at sea-level. The hottest months are April, May and June and their mean maximum temperature is 89.1°F., the highest temperature recorded being 100.7°F. December, January and February are the coldest months and they record a mean minimum temperature of 68.8°F., the extreme minimum temperature being 53.1 F. June, July, August and September are the chief rainy months, and during this period the average amount of rainfall is about 67.13″, the average annual rainfall being 70.54″.

[2] *Sylloge Algarum*, Vol. V, Myxophyceæ, 1907.

[3] De Toni (*op. cit.*, 1907, p. 108) records this alga as *Merismopedium minimum* G. Beck, while Geitler (in Rabenhorst's *Kryptogamen-flora von Europa*, XIV Band, Cyanophyceæ, 1930-32, p. 263) writes it as *Merismopedia minima* G. Beck.

[4] De Toni (*op. cit.*, 1907, p. 540) records it as *Campylonema indicum* Schmidle, but Geitler (*op. cit.*, 1930–32, p. 705) writes it as *Camptylonema indicum* Schmidle.

[5] The position of *Hapalosiphon Hansgirgi* Schmidle (syn. *Mastigocladus Hansgirgi* Schmidle) is doubtful. Geitler (*op. cit.*, 1930–32, p. 558) regards it to be a member of the Scytonemataceæ, while Kisselewa (*Jour. Russ. Bot. Ges.*, **15**, 1930) actually refers it to the genus *Scytonematopsis*.

water courses. Most of the places near the sea are low water-logged areas on which paddy is extensively cultivated. Besides, there are three small lakes and a number of small ponds between the hilly parts of the island. All these situations together with the coastal areas, helped by the heavy annual rainfall, serve as the most suitable habitats for the luxuriant growth of the Myxophyceæ, the island of Salsette being far richer than that of Bombay.

The analysis of water from different localities has shown that the algæ which grow in waters containing high percentage of salt (*i.e.*, sea-water) are (1) *Aphanocapsa litoralis* Hansg. var. *macrococca* Hansg., (2) *Anabæna orientalis* Sp. Nov., (3) *Spirulina subsalsa* Oerst., (4) *Spirulina major* Kütz., (5) *Oscillatoria sancta* (Kütz.) Gom., (6) *Oscillatoria acuminata* Gom., (7) *Lyngbya æstuarii* Liebm., (8) *Lyngbya majuscula* Harvey, (9) *Lyngbya confervoides* Agardh, and (10) *Microcoleus chthonoplastes* Thuret, while the rest are either sub-aerial or freshwater forms. From amongst the former type of algæ all the forms except *Spirulina subsalsa* Oerst. have been noticed to migrate into freshwater.

In all thirty-six forms have been recorded in this paper, out of which six have been found on moist soil, four grow on stones or rocks, three cover the bark of trees, and the rest are aquatic forms. There are three new species, six new varieties, and eight new forms.

The Myxophyceæ are generally preserved either in alcohol or in weak formalin, or sometimes in a mixture of both, with or without some glacial-acetic acid. The writer, however, kept his collections in the following solution which has been found to be an equally good preservative:—

Copper nitrate	..	0·2 gms.
Copper chloride	..	0·2 ,,
Glacial-acetic acid	..	0·5 c.c.

2% formalin solution to make up 100 c.c.

Systematic Enumeration of the Species Observed.[6]

I. Chroococcales.

Chroococcaceæ.

Genus *Microcystis* Kützing.

1. *Microcystis æruginosa* Kütz. Geitler, in Rabenhorst's *Kryptogamenflora von Europa*, XIV Band, Cyanophyceæ, 1930–32, p. 136, Fig. 59 *d*.

Lat. cell., 3·5 μ.

Habitat.—Vihar Lake. (September)

[6] The classification given by Geitler (*op. cit.*, 1930–32) has been followed in this paper.

2. *Microcystis flos-aquæ* (Wittr.) Kirchn. Lemmermann, *Kryptogamenflora d. Mark Brandenburg*, iii, Algen I, 1910, p. 75; Smith, *Ecology of the Plankton Algæ of the Palisades Interstate Park*, Roosevelt Wild Life Bulletin, Vol. 2, No. 2, 1924, Pl. 3, Fig. 13.

Lat. cell., 4 μ.

Habitat.—In ponds and tanks near Hindu temples, Khar. (July)

Genus *Aphanocapsa* Nägeli.

3. *Aphanocapsa litoralis* Hansg. var. *macrococca* Hansg. Geitler, *op. cit.*, 1930–32, p. 153. (Fig. 1, *A*).

Lat. cell., 4·5–10 μ.

Habitat.—On soil near sea-shore at Chowpaty. (August)

Genus *Aphanothece* Nägeli.

4. *Aphanothece pallida* (Kütz.) Rabenh. Geitler, *op. cit.*, 1930–32, p. 172, Fig. 78.

Forma minor. Form. Nov.

Diam. colon., up to 1·8 cm.; lat. cell., 2·5–4 μ; long. cell., up to 6·5 μ; lat. cell., cum vag., 5·5–9 μ; long. cell. cum vag., 7·5–12 μ.

Habitat.—On the bed of a stagnant rivulet, Santa Cruz (August), and Kurla (October).

This form differs from the type in the smaller dimensions of the cells.

Genus *Glœocapsa* Kütz., emend. Nägeli.

5. *Glœocapsa atrata* (Turp.) Kütz. Geitler, *op. cit.*, 1930–32, p. 186, Fig. 83 *c*.

Diam. colon., up to 30 μ.; lat. cell., 3·5 μ; lat. cell. cum vag., 6·2–12·5 μ.

Habitat.—On a rocky river-bed, Borivali. (V. N. Hate; October).

Genus *Chroococcus* Nägeli.

6. *Chroococcus pallidus* Näg. Geitler, *op. cit.*, p. 239, Fig. 116 *b*.

Forma.

Lat. cell., 5–6 μ; long. cell., 5–7·5 μ; lat. cell. cum vag., 11·2 μ; long. cell. cum vag., 14 μ.

Habitat.—On stone-walls of Mandapeshvar (Borivali) and Jogeshvari caves. (August and September)

The form differs from the type in the thick yellow-brown sheath.

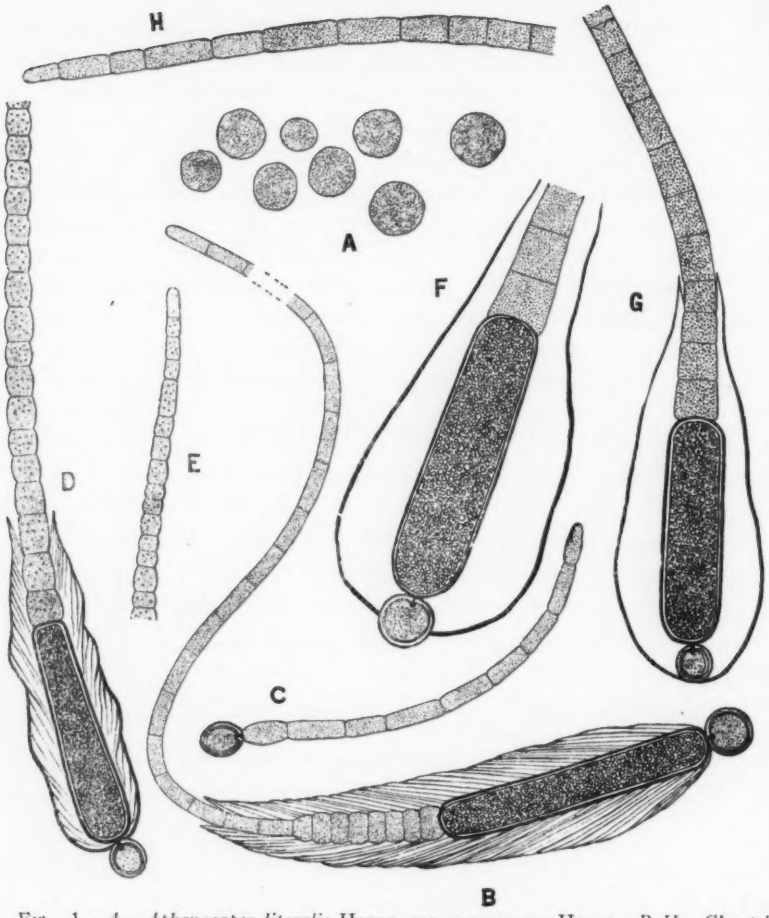

Fig. 1.—*A*.—*Aphanocapsa litoralis* Hansg. var. *macrococca* Hansg. *B–H*.—*Glœotrichia Raciborskii* Wolosz. *B*, mature and *C*, young filaments of var. *salsettense* Var. Nov.; *D*, basal, and *E*, terminal portions of the filaments of var. *bombayense* Var. Nov., *F* and *G*, basal, and *H*, terminal portions of var. *conica* Var. Nov. *A* ×910; *B* and *C* ×580; *D* and *E* ×675; *F–H* ×675.

II. Hormogoneales.

(*a*) *Nostochopsidaceæ*.

Genus *Nostochopsis* Wood.

7. *Nostochopsis Hansgirgi* Schmidle. Geitler, *op. cit.*, 1930–32, p. 479, Fig. 289.

Diam. trich., 1·2–3 μ; lat. het., 4–5 μ; long. het., 6·5–7·5 μ.

Habitat.—On rocks in a rivulet near Goregaon. (September)

(*b*) *Rivulariaceæ.*

Genus *Glœotrichia* Agardh.

8. *Glœotrichia Raciborskii* Woloszynska. Geitler, *op. cit.*, 1930–32, p. 637, Fig. 405 *a*.

Var. *salsettense*, Var. Nov. (Fig. 1, *B* and *C*).

Diam. strat., 1 cm. ; long. fil., 880 μ ; diam. trich., 5–9 μ ; crass vag., up to 2 μ ; lat. het., 7·5–14 μ ; long. het., 10–12·5 μ ; lat. spor., 12·5–15 μ ; long. spor., up to 67·5 μ.

Habitat.—Free-floating in a forest pool, Borivali. (September)

This alga differs from the type in the much bigger thallus, in the hyaline sheath showing distinct divergent stratification throughout its length, in the broader heterocysts, and in the narrower spores. It also differs from the var. *Lilienfeldiana* (Wol.) Geitler (Geitler, *op. cit.*, 1930–32, p. 637, Fig. 405 *b*) on account of the sheath being thicker and stratified, and the spores being generally thinner and slightly more elongated.

Var. *bombayense*, Var. Nov. (Fig. 1, *D* and *E*).

Diam. fil., up to 22·5 μ ; diam. trich., 3·5–6·5 μ ; lat. het., 10–12·5 μ ; long. het., 8·5–10 μ ; lat. spor., 8·5–12·5 μ ; long. spor., 37·5 μ.

Habitat.—Free-floating in a stagnant rivulet, Goregaon. (September)

The sheath in this form is brown, but the stratification is not very distinct. This variety differs from the type in the thinner trichomes, larger heterocysts, and smaller spores.

Var. *conica*, Var. Nov. (Fig. 1, *F–H*).

Diam. fil., 30–37·5 μ ; long. fil., 550 μ ; diam. trich., 5–7·5 μ ; lat. het., 10 μ ; long. het., 7·5 μ ; lat. spor., 8·5–11·5 μ ; long. spor., 55 μ.

Habitat.—Free-floating or attached to *Chara zeylanica* Wild. in a stagnant pond, Santa Cruz. (August)

This variety can be compared with var. *Lilienfeldiana* (Wol.) Geitler on account of the length of the spores and the breadth of the heterocysts. But it differs in the narrower trichomes and much thinner spores. The development of the brown sheath in this alga is sometimes so great at the base of the filament that it may altogether enclose the basal heterocyst. Moreover, the sheath thins out from base to apex, making the outer contour of the sheath more or less conical.

(*c*) *Microchætaceæ.*

Genus *Aulosira* Kirchn.

9. *Aulosira implexa* Born. et Flah. Frémy, *Les Myxophycées de l'Afrique equatoriale française, Arch. de Bot.*, iii (1929), Memoire No. 2, 1930, Fig. 318 *a* and *b*.

Var. *crassa*. Var. Nov. (Fig. 2, *A–D*).

Filaments straight or slightly curved. Sheath hyaline, stratified; at first thin, later thick. Cells usually broader than long, disc-shaped, rarely almost quadratic, with slight constrictions at the joints. Heterocysts quadratic, disc-shaped or cylindrical; single, sometimes two together. Spores not observed.

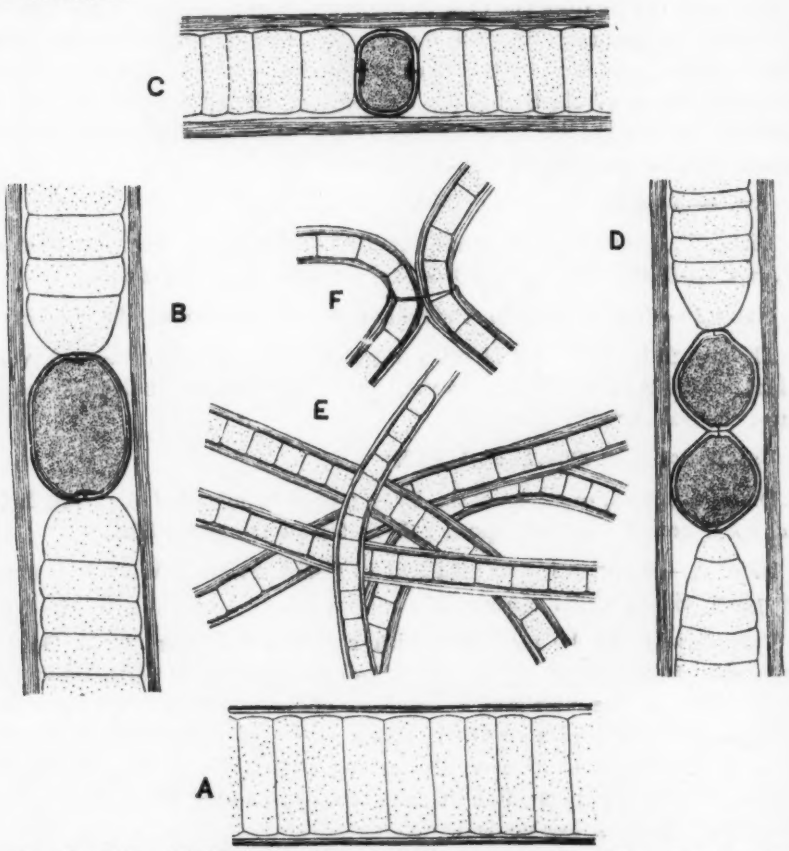

FIG. 2.—*A–D*, *Aulosira implexa* Born. et. Flah. var. *crassa* Var. Nov.; *E* and *F*, *Plectonema indica* Sp. Nov. *A* ×580; *B–D* ×675; *E* and *F* ×910.

Diam. fil., 22–25 μ; diam. trich., 16·8–18·5 μ; long. cell., 7·4–11·1 μ; lat. het., 14·8–18·5 μ; long. het., 7·4–22·2 μ; crass. vag., up to 3·7 μ.

Habitat.—Among other algæ, on a bed of a rivulet, Borivali. (V. N. Hate; January)

This variety differs from the type in the cells being usually flattened, and in the larger dimensions of all parts. The sheath becomes thicker in old filaments.[7]

(*d*) *Scytonemataceæ*.

Genus *Plectonema* Thuret.

10. *Plectonema indica* Sp. Nov. (Fig. 2, *E* and *F*).

Thallus fluffy, expanded. Filaments irregularly curved and densely entangled. Trichomes blue-green, sparingly branched, possessing both single and geminate branches. Sheath firm, at first thin and hyaline, later thick, stratified and yellow-brown; unstained with chlor-zinc-iodide. Cells almost quadratic or cylindrical, up to twice as long as broad, with slight constrictions at the joints; end-cell rounded.

Diam. fil., 12·8–14·9 μ; diam. trich., 7·4–9·2 μ; crass. vag., up to 3·7 μ.

Habitat.—On walls, along with *Chroococcus pallidus* Näg. and mosses, Mandapeshvar caves, Borivali. (September)

According to the key given by Geitler (*op. cit.*, 1930–32, p. 682) the alga comes close to *P. Tomasinianum* Born., *P. Volkensii* Schmidle and *P. Fortii* Frémy. From the first species it differs mainly in the cells being almost quadratic or cylindrical and in the sheath being not stained with chlor-zinc-iodide. It contrasts with the second species[8] on account of much thinner filaments and trichomes. From *P. Fortii* Frémy, while agreeing in the presence of almost quadratic or cylindrical cells, it differs on account of thinner filaments and trichomes, and thick stratified sheath.

The Bombay alga may also be compared with *P. nostocorum* Bornet on account of cylindrical cells, but the former species differs in the thicker filaments and trichomes.

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[7] Although *Aulosira implexa* Born. et Flah. is described to possess a thin sheath, Frémy *op. cit.*, 1930, Fig. 318*a*) has drawn it fairly thick. Probably he has drawn an older filament as is the case in the Bombay alga (*cf.* Bhâradwâja, *The Taxonomy of 'Scytonema' and 'Tolypothrix'*, Revue Algologique, Tome VII, p. 152, 1934).

[8] The description given for the cells of *P. Volkensii* Schmidle appears to be inaccurate and consequently unreliable (*cf.* Geitler, *op. cit.*, 1930–32, p. 689).

Genus *Scytonema* Agardh.

11. *Scytonema ocellatum* Lyngbye. Frémy, *op. cit.*, 1930, p. 309, Fig. 263.

Diam. fil., 12·5 μ; lat. cell., 9 μ; long. cell., 7·5 μ; lat. het., 7·5 μ; long. het., 8·4 μ.

Habitat.—Among the roots of an orchid on the bark of *Mangifera indica*; Borivali. (September)

Genus *Tolypothrix* Kütz.

12. *Tolypothrix distorta* Kütz. Var. *penicillata* (Ag.) Lemm. Lemmermann, *op. cit.*, 1910, p. 218; Geitler, *op. cit.*, 1930–32, p. 720, Fig. 461.

Diam. fil., 11–16·6 μ; crass. vag., up to 3·7 μ; diam. trich., 6·5–9·5 μ; lat. het., 6·5–10·5 μ; long. het., up to 22·2 μ.

Habitat.—In stagnant ponds and rivulets, Borivali and Santa Cruz. (August)

13. *Tolypothrix lanata* Wartm. Geitler, *op. cit.*, 1930–32, p. 718, Fig. 459 *d*.

Forma minor.

Diam. fil., 6·2–9·2 μ; diam. trich., 4·8–7·4 μ; lat. het., 4·8–7·4 μ; long. het., up to 10 μ.

Habitat.—Among other algæ on walls and also on soil, Gamdevi. (September)

The form differs from the type in the smaller dimensions of all parts, and in the absence of heterocysts in chains.

(*e*) *Nostocaceæ*.

Genus *Cylindrospermum* Kütz.

14. *Cylindrospermum muscicola* Kütz. Geitler, *op. cit.*, 1930–32, p. 816, Fig. 520 *d*.

Var. *longispora*. Var. Nov. (Fig. 3, *A* and *B*).

Lat. cell., 3·7–4 μ; long. cell., 4·5–7·5 μ; lat. het., 5–6·5 μ; long. het., 7·5–10 μ; lat. spor., 7·5–12·5 μ; long. spor., 21·5–31·5 μ.

Habitat.—On water-logged soil in rice fields, Mandapeshvar, Borivali. (September)

This form differs from the type in the slightly elongated cells, larger heterocysts and longer spores. Usually there is a heterocyst at each end of the trichome.[9]

[9] The presence of heterocysts at both ends of the trichome in *Cylindrospermum* has been described by various workers (*cf.* Bhâradwâja, "Contributions to our knowledge of the Myxophyceæ of India," *Annals of Botany*, 1933, **47**, 117).

Genus *Nostoc* Vaucher.

15. *Nostoc Hatei*[10] Sp. Nov. (Fig. 3, *C*).

Thallus almost spherical; at first attached, later free-floating; dark green. Trichomes irregularly curved and densely entangled. Cells spherical or ellipsoidal. Heterocysts single or in short chains of 2 to 5; almost spherical, rarely slightly barrel-shaped, sometimes flattened. Spores not observed.

Diam. strat., up to 2 cm.; diam. trich., 3·7–6 μ; lat. het., 3·6–5 μ; long. het., 5 μ.

Habitat.—On aquatic plants in a stagnant pond, Santa Cruz. (August)

This species resembles *Nostoc Kihlmani* Lemm. in the spherical thallus, irregularly curved filaments, and in almost spherical cells and heterocysts; but it differs from the same in the greater size of the thallus, the presence of ellipsoidal cells and smaller heterocysts, and in the absence of gas-vacuoles.

The Bombay alga sometimes possesses heterocysts in chains, and this feature, together with almost spherical heterocysts, brings it close to *Nostoc planctonicum* W. Poretzky; but it differs from this Russian species in the larger thallus, smaller heterocysts, the absence of gas-vacuoles, and in the cells being spherical or slightly ellipsoidal and not flattened and barrel-shaped.

Genus *Anabæna* Bory.

16. *Anabæna variabilis* Kütz. Frémy, *op. cit.*, 1930, p. 358, Fig. 294.

Lat. cell., 3·7–5 μ; lat. het., 5–7·5 μ; long. het., up to 9 μ; lat. spor., 7·5 μ; long spor., 8·5 μ.

Habitat.—In a road-side pool, Thana. (September)

17. *Anabæna orientalis* Sp. Nov. (Fig. 3, *D* and *E*).

Trichomes single, straight or slightly curved. Cells almost quadratic or cylindrical, rarely slightly barrel-shaped, up to almost twice as long as broad; end-cell conical with rounded apex. Heterocysts single, intercalary, cylindrical or slightly ellipsoidal with rounded end-walls. Spores one on each side of a heterocyst, ellipsoidal.

Lat. cell., 2·5–4 μ; long. cell., 3·7–4·8 μ; lat. het., 4·8–5·5 μ; long. het., 7·4–9·2 μ; lat. spor., 7·4–9·2 μ; long. spor., 14·8–16·6 μ.

Habitat.—In a rain-water pool near sea coast, Mahalaxmi. (September)

This species can only be compared with *Anabæna aphanizomenoides* Forti; both have straight or slightly curved trichomes with slight

[10] Named after Prof. V. N. Hate of Bombay.

constrictions at the joints, cylindrical cells, conical end-cells, and usually a single spore on either side of a heterocyst. The present species, however, differs in the slightly thinner trichomes, in the cylindrical heterocysts and in the ellipsoidal spores.

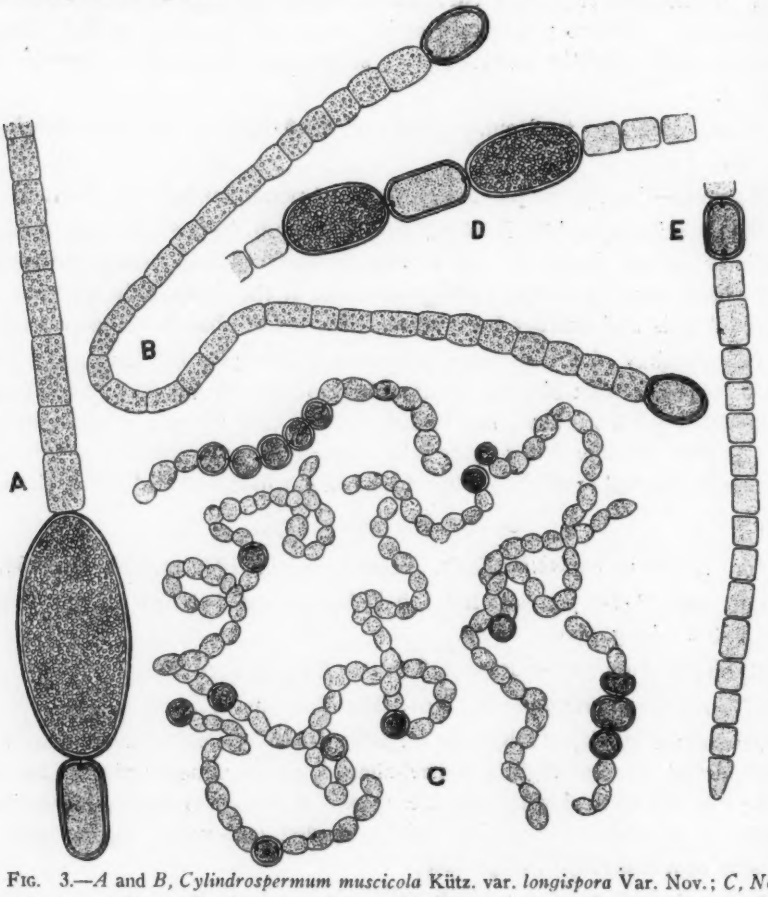

FIG. 3.—*A* and *B*, *Cylindrospermum muscicola* Kütz. var. *longispora* Var. Nov.; *C*, *Nostoc Hatei* Sp. Nov.; *D* and *E*, *Anabæna orientalis* Sp. Nov. *A* × 3175; *B* and *C* × 1820; *D* and *E* × 3175.

(*f*) *Oscillatoriaceæ.*

Genus *Spirulina* Turp.

18. *Spirulina subsalsa* Oerst. Geitler, *op. cit.*, 1930–32, p. 928, Fig. 593 *a*.

Diam. trich., 0·8 μ; lat. spir., 1·7 μ.

Habitat.—On sea-shore, Colaba. (August)

19. *Spirulina major* Kütz. Geitler, *op. cit.*, 1930–32, p. 930, Fig. 595.

Diam. trich., 1·5 μ; lat. spir., 2·5–3·7 μ; spat. inter duo. spir., 2·5–3·7 μ.

Habitat.—Among other algæ in a stagnant rivulet, Muland. (January)

Genus *Oscillatoria* Vaucher.

20. *Oscillatoria sancta* (Kütz.) Gom. Tilden, *Minnesota Algæ*, Vol. I, 1910, Pl. IV, Fig. 5.

Forma.

Diam. trich., 7·5–10 μ ; long. cell., 2–2·5 μ.

Habitat.—In a rain-water pool, Dharavi Road, Mahim. (V. N. Hate)

The form differs from the type in the thinner trichomes.

21. *Oscillatoria subbrevis* Schmidle. Geitler, *op. cit.*, 1930–32, p. 946, Fig. 601 *b*.

Forma crassa. Form. Nov.

Diam. trich., 12·5–20 μ ; long. cell., 2·5–3 μ.

Habitat.—On moist soil near a main water-pipe, Kurla. (October) ; in road-side ditch, Borivali. (August)

This form differs from the type and the Forma *major* West in the much thicker trichomes.

Forma.

Diam. trich., 7·5–10 μ ; long. cell., 1·2–1·5 μ.

Habitat.—Along with *Forma* crassa.

22. *Oscillatoria princeps* Vaucher. Frémy, *op. cit.*, 1930, p. 207, Fig. 175.

Diam. trich., 40–48 μ ; long. cell., 4–8 μ.

Habitat.—In road-side ditches, Khar and Santa Cruz. (April ; September)

23. *Oscillatoria formosa* Bory. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 33.

Diam. trich., 5–6 μ ; long. cell., 2·5–6 μ.

Habitat.—On a wooden plank near the water-pump engine, Andheri. (August)

24. *Oscillatoria acuminata* Gom. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 29.

Forma.

Diam. trich., 2·5–4 μ ; long. cell., 4–5 μ.

Habitat.—In shallow pools, Sion. (October)

The form differs from the type in somewhat smaller dimensions of the cells.

Genus *Phormidium* Gomont.

25. *Phormidium ambiguum* Gom. var. *major*. Lemm. Lemmermann, *op. cit.*, 1910, p. 127.

Diam. trich., 9 μ ; long. cell., 2·2–4·5 μ.

Habitat.—On mud, Kurla. (September)

Genus *Lyngbya* Agardh.

26. *Lyngbya æstuarii* Liebm. Tilden, *op. cit.*, 1910, Pl. V, Figs. 40 and 41.

Diam. fil., 20–24 μ ; diam. trich., 14·8–16 μ ; long. cell., 3–4 μ.

Habitat.—Free-floating in sea-water along with species of *Enteromorpha*, Santa Cruz.

Var. *tenuis*. Var. Nov.

Diam. fil., 9–12·5 μ ; diam. trich., 5–9 μ ; long. cell., 1·25–2·5 μ.

Habitat.—Along with species of *Chara* in a freshwater pond inundated by sea-water during high tide, Bhayendar. (September)

This variety differs from the type in the narrower trichomes, and in the absence of gas-vacuoles and granules by the side of cross walls.

27. *Lyngbya majuscula* Harvey. Geitler, *op. cit.*, 1930–32, p. 1060, Fig. 672 *c* and *d*.

Diam. fil., 45·6–51 μ ; lat. cell., 25–42·5 μ ; long. cell., up to 8·5 μ.

Habitat.—In a rivulet, Borivali. (October)

28. *Lyngbya confervoides* Agardh. Tilden, *op. cit.*, 1930, Pl. V, Fig. 39.

Diam. fil., 14–15·5 μ ; lat. cell., 6·5–10 μ ; long cell., 1·75–3 μ.

Habitat.—Attached to rocks at sea shore, Mahalaxmi. (January ; August)

29. *Lyngbya Allorgei* Frémy. Fremy, *op. cit.*, 1930, p. 193, Fig. 156.

Diam. trich., 4·6–6·4 μ ; long. cell., 4·6–8·3 μ.

Habitat.—On mud and stones submerged in flowing water, Borivali. (September)

Genus *Symploca* Kütz.

30. *Symploca muscorum* (Ag.) Gomont. Geitler, *op. cit.*, 1930–32, p. 1123, Fig. 730 ; Frémy, *op. cit.*, 1930, p. 129, Fig. 112 *b*.

Lat. cell., 6–8 μ ; long. cell., 6–8 μ.

Habitat.—On roots of *Ficus* Sp., near a pond, Borivali. (September)

31. *Symploca cartilaginea* (Mont.) Gomont. Geitler, *op. cit.*, 1930–32, p. 1126, Fig. 734.

Diam. fil., up to 4·5 μ ; lat. cell., 3 μ ; long. cell., up to 6 μ ;

Habitat.—On wet rocks, Malabar Hill. (September)

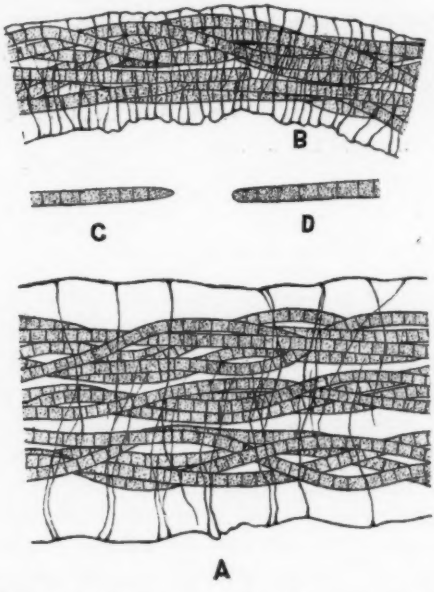

FIG. 4.—*A* and *B*, portions of filaments, and *C* and *D*, terminal portions of two trichomes of *Microcoleus chthonoplastes* Thur. All ×415.

Genus *Microcoleus* Desmazieres.

32. *Microcoleus chthonoplastes* Thuret. Geitler, *op. cit.*, 1930–32, p. 1134, Fig. 739 ; Bharadwâja, "The Myxophyceæ of the United Provinces, India, I", *Proceedings of the Indian Academy of Sciences*, II, 1935, p. 98, Fig. 2 *b* and *c*.

Forma. (Fig. 4, *A–D*).

Diam. fil., 27–75 μ ; crass. vag., 8–37·5 μ ; diam. trich., 3·7–4·2 μ ; long. cell., 4·5–7 μ.

Habitat.—Epiphytic on red algæ, Napean Sea Road and Bandra ; in freshwater pools, Santa Cruz.

This form differs from the type in sometimes possessing two to four bundles of trichomes enclosed in a common sheath, the outer limit of which is more or less crinkled and shows folds on its surface like those drawn by Fritsch

("Contributions to our Knowledge of the Freshwater Algæ of South Africa," *Trans. Roy. Soc. S. Africa*, XI, p. 360, Fig. 21) for *Microcoleus annulatus* Fritsch and Rich, and by Geitler (*op. cit.*, 1930–32, p. 1156, Fig. 764) for *Hydrocoleus Brebissonii* Kütz. Moreover, there are no constrictions at the dissepiments.

Most of this work was done in the Botanical Laboratory of the Benares Hindu University, and the author expresses his grateful thanks to Professor Y. Bhâradwâja, M.Sc., Ph.D. (Lond.), F.L.S., for his most valuable help throughout the preparation of this paper. He is also thankful to Mr. C. Bhashyakarla Rao, B.Sc., for drawing the figures.

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AN EXPLANATION TO THE OCCURRENCE OF SPORADIC CASES OF URINARY SCHISTOSOMIASIS IN INDIA.

By Col. I. Froilano de Mello.
(Nova Gôa)

Received December 26, 1935.

Up-to-date Bilharziosis has not been registered in India as an endemic disease, despite some cases imported from abroad. Stanley Kemp and Gravely[1] in an exhaustive study failed to infect the molluscs of Hyderabad and Secunderabad with miracidia of *S. hæmatobium*. I myself[2] working with *S. mansoni* imported in our territory by Mozambique troops since 1912, arrived to the following conclusions: (*a*) no inhabitant of Portuguese India showed any signal of a bilharzic dysentery in the villages occupied from 1912 to 1920 by the African infected troops (clinical and parasitological enquiry), (*b*) *Pachelabra mœsta, Indoplanorbis exustus, Melanodes pyramis* collected from various parts of our territory did not show any furcocercaria of *S. mansoni* type. Two types of furcocercariæ were however found in *Indoplanorbis exustus*, but anatomically different from those of *S. mansoni*[3]; (*c*) we failed to infect with *S. mansoni* miracidia the above-quoted molluscs. *P. mœsta* and *I. exustus* were absolutely indifferent to those miracidia; *M. pyramis*, however, showed an evident miracidial attraction. However, no actual penetration was seen, neither any stage of further sporocystic cycle.

My conclusions were that, on epidemiological and experimental grounds—at least with the molluscs above quoted—there was no fear of endemisation of Intestinal Bilharziosis in Portuguese India. Cautiously, however, I added that one should not lose the view of a fortuitous and eventual adaptation of Schistosoma to some local molluscs.

Negative conclusions were reached in British India, where Seymour Sewell[4] states the non-existence of *Bullinus* and *Hypsobia*, the universally recognised hosts of *S. hæmatobium* and *japonicum* and the existence of one single *Planorbide, P. exustus*, later on classified by Annandale as *Indoplanorbis*

exustus. It must not, however, be forgotten that Seymour Sewell found among the *Cercariæ indicæ* one resembling morphologically that of *S. japonicum* whose adult worm was not, unhappily, identified by experimental inoculations to suitable animals.[5]

* * *

Sporadic cases of Bilharziosis originated *in loco* have, however, been recorded in India, but as far as I am aware no mollusc has yet been rendered responsible as intermediate host. Leiper[6] thinks that such cases may be produced by some animal schistosome. We will quote his own words:

> " the other ss pp *bomfordi, indicum, spindalis* occur in domesticated animals, *e.g.*, horse, sheep, cattle, etc..... It is not at all improbable that they may, like *S. bovis*, occasionally invade man as incidental infections. Such infections may provide an explanation to the sporadic occurrences of cases of Bilharziosis in India."

I am not aware of any case of human urinary Schistosomiasis definitely related to a Bilharzia of animal origin. Cawston suspected once *S. spindalis* as the cause of disease in a Zulu School boy.[7] *S. spindalis* is very common in India and no human case has been reported to it. Epidemiological evidence is therefore definitely against the hypothesis of Leiper and Brumpt[8] says rightly : La realité de l'infection de l'homme par *Sch. bovis* signalée ou admise par divers auteurs reste à démontrer.

So, we must find an explanation to the occurrence of such sporadic cases and the present note is written for this purpose.

* * *

In October 1934, we had here the first and hitherto only case of Schistosomiasis. Child, 9 years old, son of an African soldier and a Goanese woman hospitalised for cystitis and dysentery and showing in urine and in fæces numerous ova of *Sch. hæmatobium*.

There is no doubt that this case is a true case of *Schistosomiasis hæmatobia* : the clinical symptoms, the good results of emetic treatment and the morphology of the ova fully justify such diagnosis.

The measurements of the ova gave the following numbers:—

(Taken on 96 ova by my pupil Miss Ciriaca Valles, before the treatment.)

No. of ova	Length (microns)	Maximum breadth
4	75	$\frac{3}{37}$ $\frac{1}{35}$
4	88	,, $\frac{1}{60}$
26	100	$\frac{1}{31}$ $\frac{1}{35}$ $\frac{12}{37}$ $\frac{3}{38}$ $\frac{3}{40}$ $\frac{6}{60}$ $\frac{1}{65}$ $\frac{1}{75}$
6	112	$\frac{1}{30}$ $\frac{1}{33}$ $\frac{4}{37}$
1	115	38
2	120	$\frac{1}{37}$ $\frac{1}{60}$
13	125	$\frac{1}{32}$ $\frac{1}{33}$ $\frac{1}{35}$ $\frac{3}{37}$ $\frac{3}{50}$ $\frac{1}{60}$ $\frac{1}{62}$ $\frac{1}{70}$ $\frac{1}{80}$
1	130	75
1	135	75
2	145	$\frac{1}{75}$ $\frac{1}{68}$
16	150	$\frac{1}{50}$ $\frac{4}{60}$ $\frac{1}{62}$ $\frac{1}{65}$ $\frac{3}{70}$ $\frac{1}{72}$ $\frac{4}{75}$ $\frac{1}{80}$ $\frac{1}{90}$
1	155	75
3	160	$\frac{1}{60}$ $\frac{2}{75}$
6	170	$\frac{3}{80}$ $\frac{1}{63}$ $\frac{2}{75}$ $\frac{1}{100}$
6	175	$\frac{1}{60}$ $\frac{3}{70}$ $\frac{1}{80}$
4	180	$\frac{1}{60}$ $\frac{1}{70}$ $\frac{2}{75}$

(Taken in 100 ova by my pupil Manuel da Veiga Coutinho during treatment, to see whether there was some alteration suggesting the hypothesis of abortion put forward by C. França[9].)

No. of ova	Length (microns)	Maximum breadth
1	60	30
3	70	$\frac{1}{20}$ $\frac{1}{30}$ $\frac{1}{40}$
2	75	$\frac{2}{40}$
10	80	$\frac{3}{25}$ $\frac{2}{30}$ $\frac{2}{35}$ $\frac{1}{40}$ $\frac{2}{45}$
9	85	$\frac{2}{25}$ $\frac{1}{30}$ $\frac{4}{35}$ $\frac{2}{45}$
14	90	$\frac{1}{20}$ $\frac{2}{25}$ $\frac{6}{30}$ $\frac{3}{35}$ $\frac{1}{40}$ $\frac{1}{45}$
12	95	$\frac{1}{25}$ $\frac{3}{30}$ $\frac{2}{35}$ $\frac{4}{40}$ $\frac{2}{50}$
6	100	$\frac{1}{25}$ $\frac{2}{30}$ $\frac{1}{35}$ $\frac{1}{40}$ $\frac{1}{45}$
13	105	$\frac{1}{30}$ $\frac{4}{35}$ $\frac{4}{40}$ $\frac{2}{45}$ $\frac{2}{50}$
8	110	$\frac{1}{30}$ $\frac{1}{35}$ $\frac{4}{40}$ $\frac{1}{55}$ $\frac{1}{70}$
9	115	$\frac{1}{30}$ $\frac{1}{40}$ $\frac{2}{45}$ $\frac{2}{50}$ $\frac{2}{55}$
3	120	$\frac{1}{55}$ $\frac{2}{60}$
3	125	$\frac{1}{25}$ $\frac{2}{35}$
2	130	$\frac{1}{35}$ $\frac{1}{40}$
3	135	$\frac{1}{30}$ $\frac{1}{55}$ $\frac{1}{70}$
1	150	65
1	165	45

How was this case originated?

Unless we admit some other mechanism of transmission, we are obliged to accept that some local mollusc was infected and that its cercariæ did by their turn infect the child.

And as the case is a single one we must admit that the responsible mollusc is a very rare one or that the infection occurred only *eventually, accidentally* in a mollusc showing some affinities to those miracidia.

Such an hypothesis of an *eventual adaptation* is not a rarity in the history of parasitism and even in the life cycle of human Schistosomes, if we consult the literature, valuable information can be collected on this point of view, suggesting the division of the mollusc hosts into *habitual* or those in whose bodies the bilharzial life cycle always occurs, *secondary* or those where such life cycle occurs but not so frequently, and *eventual* or those who having shown an evident miracidial attraction, often followed by penetration, have not, however, given in the hands of the authors a definite final phase of sporocysts and cercariæ and are therefore only suspected as able to act as intermediate hosts. On a medical point of view it is much more reasonable to support this last view than to state that probably those rare human bilharzial sporadic cases must be of an animal origin in countries where the suspected animal bilharzioses are widely spread and could naturally produce more human cases.

Following these lines we find :

Schistosomum hæmatobium.

Habitual hosts.—Bullinus dybowsky, innesi (all classified by Baylis as *Bullinus truncatus* Andouin, 1826);[10] Physopsis africana, globosa, Isidora (Physopsis) ovoidea,[11] Physopsis conicum,[12] Planorbis dufourii.[13]

Secondary hosts.—*Limnæa natalensis* (Porter[12,14]), Bullinus brochii (Anderson[15]), Melania nodocincta (Dye[16]), Melanoides tuberculata (Gopsill[17,18]).

Eventual hosts.—Limnæa truncatula (Leiper in Raynaud et Léger[19], miracidial attraction), Pyrgophysa forksali (*id.*[19]), Planorbis boissyi (*id.*[19]), Planorbis albus (Roubaud *id.*[20]), Lymnæa limosa (*idem* with penetration in one specimen[20]), Bullinus strigosus (Léger[21] mirac. attraction), Pyrgophysa dunkeri (*id.*[21]), Lymnæa africana (*id.*[21]), Melania tuberculata (*id.*[21]), Physalopsis globosa (*idem* with penetration França[9]).

Schistosomum mansoni.

Habitual hosts.—Planorbis (Planorbula) boyssii, Pl. (Planorbis) Pfeifferi, Pl. (Planorbina) guadaloupensis, Pl. (Planorbina) centimetralis, Pl. (Planorbina) antiguensis, Physopsis africana, Bullinus tropicus.

Secondary hosts.—Melanoides tuberculata (Gopsill 18), Planorbis sp. (closely allied to P. sudanicus Marten) (Dye[16]).

Eventual hosts.—Planorbis ferrugineus (Lutz[22] mirac. attraction with penetration but without the final cercaria phase), Pl. tenagrophylus[22], some Physa[22,19], Pl. cultratus (Iturbe and Gonzalez cit.[19]), Ampullaria luteostoma (Iturbe and Gonzalez cit.[19]), Melanodes pyramis (mir. attraction alone de Mello[2]).

Schistosoma japonicum.

Habitual hosts.—Onchomelania (Hypsobia) nosophora, O. formosana,[6] O. hupensis,[6] (Prososthenia) Blanfordia quadrasi (Tubangui[23]).

* * *

After these preliminary remarks, we will report the experiments made in our laboratory in order to find out the possible molluscan vector responsible for our case. The snails were collected in the villages where the infected child was living, kept under observation during 48 to 72 hours for examination of any furcocercaria infesting them *in natura*, and only those recognised free, submitted to further experiments of miracidial attraction and afterwards transferred to a small tank where during 2 months the residuum of the child urine containing living ova was put daily for realising as far as possible a natural mode of infection of those snails.

The following are the results of the experiments :

Pachelabra mæsta.—No miracidial attraction. No furcocercaria or sporocyst after 2, 3 and 4 months of observation.

Indoplanorbis exutus.—Idem.

Melanoides tuberculatus.—The child being cured at the time when this snail came to our laboratory, experiments of infection could not unhappily be carried on. It must be emphasised that *M. tuberculata* has been incriminated by Gopsill as responsible vector both of *S. hæmatobium* and *S. mansoni* in the Lower Shire District. One of our *Melanoides* showed *in natura* one furcocercaria not alike to that of *S. hæmatobium.*

Limnæa luteola Lamarck *f. pinguis* Dohrn.—Miracidial attraction very active. Rarely, evident penetration, seen sometimes after four hours of observation. No sporocystic or cercaria stage seen after 2, 3 and 4 months of the infection in the tank.

Conclusions.

1. Clinical, epidermiological or experimental facts do not authorise the conclusion that urinary Bilharziosis may become endemic in Portuguese India.

2. However, one definite autochthonous case of human urinary Bilharziosis has been registered in the village of Valpoy where from 1912 to 1934 were living African troops infected with these trematodes.

3. Unless some other mechanism of infection be discovered, it is reasonable to believe that some local mollusc has conveyed the infection.

4. This intermediary mollusc cannot be an *habitual* host—as the epidemiological facts are entirely against such an hypothesis—but a snail in which this infection occurs rarely and in some way *accidentally*.

5. On this point of view we propose the division of the vectors of Schistosomiasis into 3 groups: *habitual*, *secondary* and *eventual*, according to the evolutive cycle occurs *in natura* and frequently, *in natura* or experimentally but rarely and at last, as *eventual* those who having shown a definite miracidial attraction, often followed by penetration, have not, under experimental conditions, demonstrated the final phase of sporocyst or cercaria.

6. We believe that such *eventual* hosts are responsible for the sporadic cases of Bilharziosis occurring in an indemn area, after the import of this disease from abroad.

7. Among such molluscs two species are found in Valpoi: *Melanoides tuberculatus* already incriminated by Gopsill, and *Limnæa luteola* Lamarck *f. pinguis* Dohrn, where an evident miracidial attraction often followed by penetration was definitely seen.

We hope that further studies, made in India, whenever fresh Bilharzial material be available, will confirm or infirm the present hypothesis.

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THE BLOOD GROUPS IN PORTUGUESE INDIA.

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Received November 20, 1935.

The blood groups may be considered as a differential character among the somatic characteristics of the human body, under the ethnological point of view.

The blood grouping represents an excellent test to the classification of the races of mankind.

It is based on the hemoagglutination, phenomenon which is a special quality, of blood yet not more than one special quality, hence one somatic characteristic only.

In the whole realm of anthropological distinctions, as wisely proved the Prof. Suk, of Brno, this one quality of blood has been extensively used with regard to its supposed racial significance.

It is true also, that, there is at present, other blood qualities not so well known because the blood grouping itself is not yet completely characterised and fully standardised.

According to the opinion of Prof. Thomsen, of Copenhagen, the existence of two A genes can be served to explain anomalous differences found in certain racial groups.

As the craniological characters only are not sufficient to define thermic groups of the humanity, similarly the blood grouping, when considered isolately, is unable to characterise any family of the mankind.

Thus the results of the hemoethnological researches must be used as a supplement to the other anthropological features and measurements.

But, it must be emphasised, that there is no unanimity of opinion among the authors as far as concerns the interdependence of racial classification based on blood grouping and on morphological features.

It is also observed and demonstrated that some comparisons between blood groups and the usual physical traits, at present considered as suitable for racial classification, do not show any co-relation.

Numerous ethnic samples with their respective blood groups ethnographically quite separated, show among them a great identity or many resemblances ; and on the other side, human groups living in the same country and showing somatic equality of the anthropometric data do not show any identity with regard to their blood group percentages.

Concerning the blood grouping problem in the Portuguese India this paper is the first contribution for its scientific resolution.

My researches on this anthropological subject were made in 400 Maratha soldiers of the Gôanese garrison, and I have adopted for these investigations the Beth-Vincent's method, using the serums manufactured in the Institut Pasteur of Paris.

I have measured 162 of those Marathas in my laboratory at the Medical College of Nova-Gôa.

The percentages of the blood groups which I have observed among them are the following :

	Number of subjects	Percentages
A group	107	26,75 %
B group	136	34,00 %
A B group	40	10,00 %
O group	117	29,25 %

Hirszfeld's Index .. 0,84.

This index is higher than those found by the Drs. Hirszfeld, Malone, Lahiri, Bais and Verhöef.

Dr. Hirszfeld has found the index 0,56 in the Indian soldiers, observed by him at Salonica. Those soldiers, in number 1,000, were chiefly Sikhs and Goorkhas fighting against the Turkey army of Galepoli. If the Sikhs are of the white race the Goorkhas, inhabitants of the Nepal, belong to the yellow race.

It seems to me that the Goorkhas were the cause of the low index attributed by Hirszfeld to the Indian troops of the English army of Dardanels.

The second study of the blood groups of Hindus has been made in the Dutch Indies by the Drs. Bais and Verhöef.

These researchers have examined 348 Kligalese or Tamils, immigrants proceeding from Ceylon and working in the sugarcane plantations of Sumatra. They found among them the following percentages: A group—23,0%; B group—31,6%; AB group—7,5% and O group 37,9%, resulting an index of 0,72.

This index is higher than that of 0,56 as we have seen, found by Dr. Hirszfeld, but lower than that of 0,84 which I have found here as the index of the Gôanese Marathas.

The third series of blood grouping investigations in India are the researches of Drs. Malone and Lahiri at the Pasteur Institute of Kasauli.

This large inquiry is also imperfect because it was not accompanied by the somatic measurements as the former ones were made without the support of the anthropometric data.

These authors—two microbiologists but not anthropologists—have observed 3·764 individuals belonging to all the principal ethnic groups of the Indian population and they write on this subject the following:

"If[1] the figures given in the column race index be examined, it will be seen that there is little relation between the race index and the anthropological characters, thus, for example, with the Dravidians and the Indo-Aryans, two totally different types with regard to physical characteristics.

"The Dravidian type has the highest frequency and the Turko-Iranian the lowest of B; the frequency of B decreases as we travel up the Ganges valley to the North-West Frontier; the Hindus of the United Provinces standing in an intermediate position between the Dravidians and Indo-Aryans in this regard.

"These findings are quite in accordance with what might be expected from the views expressed by Risley with regard to the origin and relationship of these types.

"The same order holds good for O and AB, the Dravidians having the lowest percentage of O and the lowest of AB. The frequency of A is also highest in the Dravidian and lowest (except for Hindus of the United Provinces) in the Turko-Iranian.

"The low frequency of B in those three races of India, with the control of British soldiers, confirm the findings of other workers with regard to the Asiatic peoples in general."

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[1] "Distribution of blood groups in India," by Drs. Malone and Lahiri in *The Indian Journal of Medical Research*, 1929, 966-967.

And the same workers, trying to justify the low frequency of A amongst Hindus of the United Provinces, have written the following:

"With[2] regard to the low frequency of A amongst Hindus from the United Provinces, a figure between those obtaining for Indo-Aryans and Dravidians might have been expected since the Hindus are an admixture of the two above races, but this is not the case; no explanation of the discrepancy can be offered at present."

After concluding my present work, I came with an excellent work on the same subject by my colleague Prof. Pacheco de Figueiredo[3] *Contribution to the study of blood groups in the Indians* (*Christians and Hindus of Gôa*). For this purpose he examined 509 persons, 309 Christians and 200 Hindus and obtained the following results, which emphasise the results obtained by me, being more or less equals:

Christians			Hindus		
Groups	Examined	Percentages	Groups	Examined	Percentages
O	96	31,06	O	67	33,5
A	70	22,65	A	49	24,5
B	94	30,42	B	62	31,0
AB	49	15,85	AB	22	11,0
Index of Hirszfeld 0,83			Index of Hirszfeld 0,84		

He draws the following conclusions:

"It would be too early to draw any conclusion whatever from this work. However, it should be noted that, on the whole, the percentages of the different groups—Christians and Hindus—of Gôa is very much similar; preponderating the O group and being followed according to the frequency by the groups B, A and AB, which fact is to be noted also in the Hindus of Ceylon (Bais and Verhöef). But, while in these the Hirszfeld index is inferior (0,71), that of the Christians (0,83) and of the Hindus (0,84) of Gôa

[2] *The Indian Journal of Medical Research*, 1929, 966-967.

[3] *Arquivos da Escola Medico-Cirurgica de Nova Goa*, 1935, Serie A, Fasc. 10°, pp. 2032-2036.

is almost equal to that obtained by Prof. Germano Correia in the Mahrattas (0,84)."

It seems to me that there are some facts inexplicable in the calculation of the Hirszfeld's index among the principal ethnic groups of the Indian population.

How can we consider the absolute equality of the same index between the Indo-Aryan and the Dravidians as they have been calculated by Drs. Malone and Lahiri ?

It is impossible to admit that the index of the Hindus of the United Provinces can be lower than that of the Dravidians, because these inhabitants of India are quite more different from the white ethnic type than the Hindus of the Central Provinces, where we can find frequently human samples of the caucasian somatic type.

In other side, the blood grouping researches of Drs. Hirszfeld, Bais and Verhöef concerning Indian inhabitants without race distinctions and without the anthropometric data, cannot be considered scientifically as true and valuable.

Thus, in this condition, what can be done ? A large anthropohematic inquiry, using the same methods and serums, made by anthropologists *double* of hematologists, researching simultaneously in different provinces of the Indian Empire, but always under the directives of a harmonic plan. All those blood grouping investigations must be accompanied by the somatic measurements and informations about the race, the caste and other ethnographic data.

ON A TRYPANOSOME FOUND IN THE BLOOD OF THE INDIAN FRESH WATER FISH *CLARIAS BATRACHUS* LINN.

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Received January 6, 1936.

The Indian fishes have rarely been studied on the point of view of their blood parasites. As far as our informations go only a paper by Lingard has been written on this subject.[1] The blood parasites recorded on the Indian fishes are[2]:

Barbus carnaticus. Trypanosome (unnamed). Lingard 1903.

Gobius giuris. Trypanosome (unnamed). Patton 1908. (First recorded by Castellani and Willey 1905 in Ceylon.)

Macrones seenghla. Trypanosome (unnamed). Lingard 1904.

Ophiocephalus striatus. Trypanosome (unnamed). Lingard 1903.

Trichogaster fasciatus. Trypanosome (unnamed). Lingard 1904, Plimmer 1914.

Description of our Trypanosome.

Some specimens of *Clarias batrachus* (Portuguese name: *peixe bigodeiro*, Konkani: *tigur*), kindly identified by Dr. Hora, from the Zoological Survey of India (Calcutta) have been examined. Two of them showed trypanosomes, not very abundant, as in each slide not more than half a dozen parasites could be available. General morphology typical of a trypanosome. Anterior extremity pointed, rostrum like. At some distance behind its point a very conspicuous kinetonucleus is found under the form of a very compact granule, stained dark violet by Romanowsky derivates and lodged in the anterior part of a very fine, oval alveole (Fig. 1). The macronucleus is oval, its long axis parallel to the axis of the parasite and composed of some chromatic granules packed up together and where no distinct centriole is visible. From the kinetonucleus starts the axoneme of the undulating membrane, under the form of a merely blue sinuous line at Leishmann stain, and chromatic violet at May Grunwald Giemsa. At the posterior extremity this axoneme becomes a free flagellum,

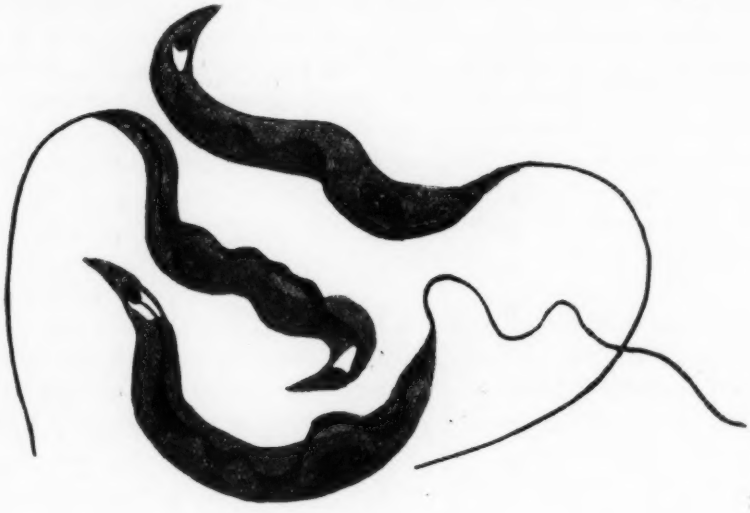

FIG. 1.

often as long as the body, and abruptly cut at its distal end, not terminating therefore by an effilated point. The macronucleus is sub-central and situated at the limit of the middle and posterior thirds. The protoplasm is irregularly vacuolated, showing seldom on the middle zone, in front of the nucleus, irregularly scattered minute volutine granules, and often in the same middle zone a kind of longitudinal striation (myonemmes ?). All the parasites studied are monomorphic and excepting to the measurements of the flagella do not show any variations at all. Longitudinal division rarely seen.

The measurements taken on 25 individuals gave the following number (see Table on p. 122).

In the list of Wenyon[2] we find that some species of *Clarias* have been found harbouring blood trypanosomes :

Clarias angolensis : Trypanosome (unnamed). Dutton, Todd and Tobey 1906, Congo.

This trypanosome has the following characters[3] : Three types ; small, median and long ones, all variations of the same species, and possessing a large four-lobed blepharoplast. There is also a constant clear space about the nucleus. The *small type* has a faint staining nucleus situated more anteriorly than in the other two types, and with its long diameter parallel to that of the body. Protoplasm reticular with few violet stain granules

No.	From the ant. extr. to the kinetonucleus	From the kinet. to the ant. border of the nucleus	Long axis of this nucleus	From the post. border of the nucleus to the end of the cytoplasm	Free flagellum	Breadth
1	1,5	12	2	5	22	1,5
2	1,5	16	2,5	6	16	1,5
3	1	13	2	6	18	1
4	2	8	2,5	3	14	1,25
5	1,5	13	1,5	5	20	1
6	2,5	16	2,5	4	21	1
7	1,5	12	2	5	19	1
8	3	19	2	5	20	1
9	3,5	10	2,5	8	18	1 5
10	1	17,5	2,5	7	15	1,25
11	3,5	17	2,5	3	16	2,5
12	1	18	1,5	8	22	2
13	2,5	15	2	8	17	2
14	3	20	2	2	20	2,5
15	1,5	16	2	8	16	1,25
16	2,5	17,5	2	8	16	1,5
17	2	10	3	13	15	2
18	1	11	2	5	13	2,5
19	2	3	2,5	7	15	2,5
20	3	14	2,5	7	9	1,5
21	2	20	2	4	11	1
22	1	13	2,5	5	22	2
23	1	13	3	7	22	2,5
24	2	6	1,5	4	15	1,9
25	1	15	3	5	32	1

Superficial longitudinal striations to the number of five near the nucleus. *Median type* darker staining, large oval or round blepharoplast, situated at or from 1 to 1,5 microns from the point. Flagellum longer than in small type. Nucleus round or oval, its long diameter at right angles to that

of the body and occupying the whole width of the parasite. Superficial longitudinal striations to the number of seven near the nucleus. *Long type* with tapering extremities, comparatively short flagellum, one to four vacuoles just behind the blepharoplast. Eight superficial longitudinal striations. Total length 34,5 ; 45 ; 61,5 respectively.

From the descriptions and the illustrations of the authors (their Plate XXX), our parasite is not the same as that of *Clarias angolensis* as it differs specially by the uniformity of its monomorphic appearance.

Clarias anguillaris.—Trypanosoma toddi Bouet 1909, Wenyon 1909, African Soudan.

We were not able to read the paper of Bouet and in Wenyon[4] we find only a record of this parasitism in *C. anguillaris*. Laveran and Mesnil state that it should be identified to the trypanosome of Dutton, Todd and Tobey.

Clarias gariepinus.—Trypanosome (unnamed). Fantham 1919, South Africa.

Clarias macrocephalus.—Trypanosoma clariæ Montel 1905, Cochin-China, Mathis and Leger 1911, Tonkin.

The characters of this trypanosome are taken to Laveran and Mesnil[5] who say that the description of Montel has been completed by Mathis and Leger. Two varieties of this parasite have been found : *Var parva*—narrow, pointed ends, protoplasm not vacuolated, showing granulations, specially on the anterior part. Voluminous oval nucleus, situated on the middle ; with its long axis parallel to the axis of the parasite, and constituted by irregularly disposed chromatic grains. Round blepharoplast near the anterior point. Free flagellum having nearly the length of a quarter of the total length of the parasite. Measurements : Total length 39, breadth 2,75, free flagellum 9. *Var magna*—posterior extremity gradually pointed, anterior one abruptly blunt before the blepharoplast, often bifid. Protoplasm irregularly vacuolated, a series of vacuoles behind the centrosomes being fairly constant. Longitudinal striation in the nuclear region. Chromophil granules on the posterior fifth ; often disposed in parallel lines. Oval nucleus, central, with its long axis generally perpendicular to the long axis of the parasite. Blepharoplast staining deep rose. Free flagellum of the same size as in *Var parva*. Measurements : total length 64, breadth 5, free flagellum 11.

Laveran and Mesnil believe that this trypanosome is very similar to that of *Clarias angolensis*.

(*N.B.*—In the description the kinetonuclear extremity is stated as posterior and the flagellate one as anterior, just the contrary of our designations.)

Clarias sp. Trypanosoma (unnamed). Zupitza 1909, Cameroon. In Laveran and Mesnil[5] we find : Trypanosomes have been found by Zupitza in two species of fishes : *Periophtalmus Kœlreuteri* and (probably) one *Clarias*. The author describes a series of forms 40–60 microns long, differing specially by their breadth which goes from 1,5 microns to 9, with all intermediate types. Blepharoplast almost terminal.

Discussion.

If we resume now the data, which we were able to collect, we see that fishes of the genus *Clarias* are parasitised by a trypanosome. That of *C. angolensis* shows three variations, that of *C. anguillaris* has been identified by Laveran to the former, despite the classification of Bouet who, having certainly seen the morphology described by Dutton, Todd and Tobey, found himself, however, authorised to create the sp. *T. toddy*. *T. clariæ* of Montel, much ressemblant to the trypanosome of Dutton, Todd and Tobey shows two varieties, the trypanosome of Zupitza is extremely polymorphic. The trypanosome we are describing now is characterised by its remarkable monomorphism.

On morphological grounds we believe that all those trypanosomes (excepting that of Fantham on which we have no information) are only varieties of a single species which, at most and only as varieties, could be described under special designations.

In our trypanosome the constancy of its morphology and staining reaction, the uniform constitution of the blepharoplast, the oval form of the macronucleus and the constancy of its situation on the limit of the middle and posterior third of the body as well as the different sizes of the free flagellum enable us to describe this trypanosome as *Trypanosome clariæ* Montel (1905) *var. batrachi* var. nov.

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SUBULURA HINDI N.SP. A NEW NEMATODE PARASITE OF *SCIURUS PALMARUM*.

By M. B. Mirza.
(From the Zoological Laboratories, Muslim University, Aligarh, U.P.)

Received January 27, 1936.

Six specimens of *Sciurus palmarum* were captured in the writer's house and sacrificed. All of them were found to be infected with *Subulura* species and a large number of male and female worms was obtained from the cæcum. In one case only, a nodule was cited below the dermis of a squirrel and on opening this a larva of *Physaloptera* sp. (described by the writer of this paper in *Zeit. f. Parasiten*, Bd. 6, Ht. 5, 1934) was taken out.

Morphology.—Body white filiform, cuticle, finely striated transversely. Lateral alæ relatively short reaching to the level of the posterior part of œsophagus. Mouth hexagonal, buccal cavity small 0·05 mm. deep, cylindrical with chitinous walls and with 3 teeth at entrance to œsophagus.

Males.—8·26 × 0·24 mm. to 13·06 × 0·41 mm. The following measurements have been taken in the longest male specimen. The length of œsophagus exclusive of bulb is 1·48 mm.; the œsophageal bulb is pyriform 0·24 × 0·19 mm. The lateral alæ extend to a distance of 1·14 mm. from the anterior end and are 0·061 mm. in width; they are finely striated. The tail is slender and ends in a point. The cloacal aperture lies at a distance of 0·244 mm. from the posterior end. The sucker is elliptical without chitinous ring. It is 0·68 mm. anterior to the cloacal aperture. Gubernaculum is 0·18 mm. long. The spicules are equal, curved 0·91 mm. long by 0·021 mm. broad, pointed posteriorly and infundibuliform anteriorly. Caudal alæ are present. There are 11 pairs of papillæ: three pairs of large ventral papillæ; two pairs of large adanal papillæ; six pairs of post-anal papillæ, of which two large pairs are near the cloacal aperture, followed by a large lateral pair and a small ventral pair, and these by two moderately large ventral pairs near the end of the tail (Fig. 1).

Females.—17·01 × 0·49 mm. to 23·37 × 0·52 mm. The following measurements have been taken in the shortest female specimen. The vulva lies at a distance of 5·69 mm. from the anterior end, slightly anterior to the middle of the body. The anus is at a distance of 1·25 mm. from the tip of tail. Eggs 0·07 × 0·06 mm., embryonated at time of deposit.

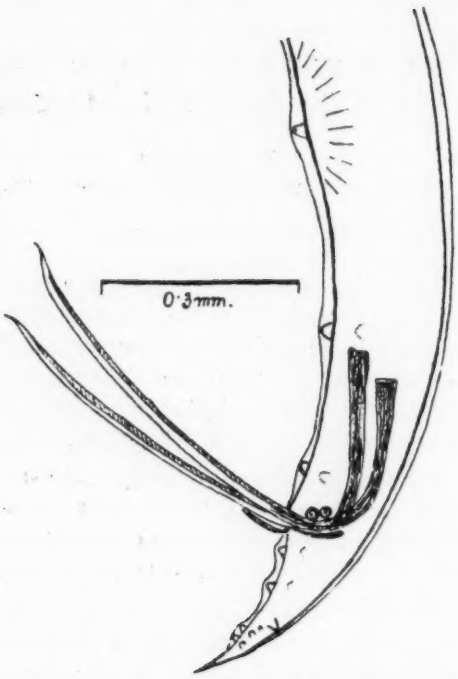

FIG. 1. *Subulura hindi* n.sp., Posterior extremity of male, lateral view.

There are only seven members of this group, recorded from birds, which possess 11 pairs of papillæ : *Subulura galloperdicis* Baylis and Daubney, 1922 ; *S. curvata* von Linstow, 1883 ; *S. strongylina* Rud., 1819 ; *S. olympioi* Baretto, 1919 ; *S. halli* Baretto, 1919 ; *S. seurati* Baretto, 1919 ; *S. suctoria* Railliet and Henry, 1912.

S. uncinata (Rudolphi, 1819) Hall, 1916, and *S. boueti* (Gendre, 1911) Travassos, 1913, which according to Hall have no morphological differences, also possess 11 pairs of papillæ distributed in the same way as in *S. hindi* n. sp. but the former possess one and only one spicule.

S. andersoni (Cobbold, 1876) Railliet and Henry; 1914, has been described by Cobbold to be parasitic in the cæcum of *Sciurus* species in North-East India. Cobbold's study of this species is not adequate but from the insufficient description given by him his species has no similarity whatsoever with *S. hindi*.

The number and disposition of the caudal papillæ in the male, the length of the spicules and the position of the vulva in the female are salient

characters which serve to distinguish *Subulura hindi* from the other members of this group.

The specimens have been deposited in the Museum of the Zoological Laboratories, Aligarh Muslim University. No. 952, type slides 945–951.

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CONTRIBUTIONS TO THE OSTEOLOGY OF THE OPHIDIA.

I. The Endoskeleton of the So-called 'Blind-Snake', *Typhlops braminus* Daud.

By Beni Charan Mahendra.

(From the Department of Zoology, St. John's College, Agra.)

Received December 29, 1935.

(Communicated by Prof. C. R. Narayana Rao, M A.)

1. Introduction.

The present communication is the first of a series, designed to supplement our knowledge of the osteology of the Ophidia. A few years ago, the author (Mahendra, 1932) submitted a short paper "On the Genetic Relationships of *Ophidian* Families" to the Indian Science Congress, and he tried to show—amongst other points—that the family *Uropeltidæ* is closely allied to the families *Typhlopidæ* and *Glauconiidæ*, and that the family *Boidæ*, contrary to Boulenger's belief (1893), cannot be regarded as the most primitive family of snakes. Since that time, he has made much further study of this question of affinities, and although the facts have remarkably borne out his conclusions, he has felt it necessary to add more details to the already known osteology of the Ophidia. In the present series, he selects representative species from each family and describes their endoskeletons in order to supplement our knowledge about them, reserving discussional remarks for the end.

Typhlops braminus, the species selected first for an osteological examination, comprises small, worm-like burrowing snakes, fairly common all over India and found also in Southern China, the Malay Peninsula and Archipelago, Arabia, Africa south of the Equator, Madagascar, the Islands of the Indian Ocean and Mexico. When full grown, this species reaches a length of 7 inches (Boulenger, 1890) or a little more (*vide infra*). Most laymen regard it as the young one of a snake rather than as an adult creature and are afraid of it, even though its minute mouth and microscopic teeth incapacitate it for inflicting a bite on human beings.

I am especially indebted to Prof. N. M. Antani for much help in the preparation of the photomicrographs, and to the authorities of St. John's College for the generous encouragement they give me in my work. I am also indebted to the Director, Zoological Survey of India, for lending me the necessary journals in this connection.

2. *Material and Technique.*

Four properly identified specimens of *Typhlops braminus* were used for this study, and two of them, at any rate, were quite adult. The respective lengths of these specimens were 3.5, 5.5, 6.25 and 7.5 inches. The last length exceeded the maximum length recorded for this species by Boulenger (1890) by half an inch. All the specimens were treated according to what is known as the " alizarin " method. After they had been fixed for 2 days in 90 per cent. alcohol, they were transferred to a 1 per cent. solution of potassium hydroxide, in which they were kept for 3 to 4 days. They were then immersed in the following solution in order to stain the bones :

1 part Alizarin sicc. (Grübler)
1,000 parts : 1 per cent. solution of potassium hydroxide.

The stain from all the tissues *other* than the skeletal was removed by placing the specimens in a 1 per cent. solution of potassium hydroxide, and the stained individuals were stored in glycerin till needed for study. Before actual examination of the osteological features, specimens were dissected under a binocular microscope so as to remove extra tissues from the bones, and in all cases careful study was made under the lower powers of a microscope.

3. *The Skull.*

The recent researches of Haas (1930) on the families *Typhlopidæ* and *Glauconiidæ* contain so detailed an account of the skull of the species *Typhlops punctatus* that it is hardly necessary to describe the skull of *T. braminus* in detail. A few remarkable features, however, deserve mention.

The Parietals (Fig. 1, D, *p*) are paired even in the full-grown individual, and they are widely separated from each other, as well as from the frontals in front and from the exoccipitals behind. The presence of paired, separate parietals in the adult *T. braminus* is an exceptional character in the *Ophidia* which is not generally known. Gadow (1901), for example, says that in the Ophidia "the parietals are *always*[1] fused into a large unpaired bone, which generally forms a sharp crest and partly overlaps the occipitals." Sedgwick (1905) mentions that "the parietal is *unpaired*[2] and sends down lateral processes which articulate with the basisphenoid." Williston (1925) thinks that in the Ophidia or Serpentes "the parietals are always fused" (p. 72).

[1] *Italics are mine.*—B.C.M.
[2] *Italics are mine.*—B.C.M.

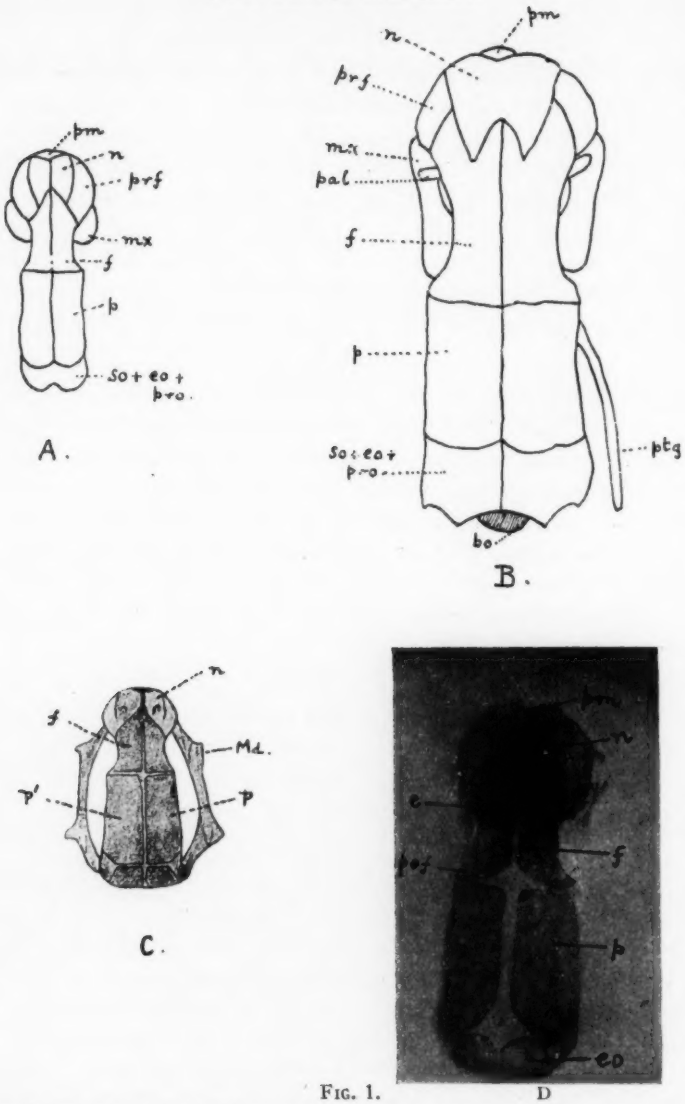


FIG. 1.

Dorsal aspect of the skull of *Typhlops braminus*, as drawn by various authors.

A. Jan et Sordelli (1870-1881). *B*. Haas (1930). *C*. Mookerjee and Das (1932). *D*. Mahendra (1935; photomicrograph).

bo, basioccipital; *eo*, exoccipital; *f*, frontal; *mx*, maxilla; *n*, nasal; *p*, parietal; *pal*, palatine; *pm*, premaxilla; *pof*, postfrontal; *prf*, prefrontal; *pro*, prootic; *ptg*, pterygoid; *P*, right parietal; *P'*, left parietal; *so*, supraoccipital; *Md*, mandible.

Note the absence of postfrontals and postorbitals in Figs. 1-3, the fused supraoccipital, exoccipital and prootic elements in figs. A and B, and the inaccurate appearance of the cleft in fig. C.

In this connection, my attention has been drawn to a short note by Mookerjee and Das (1932), in which they claim to be the first discoverers of the presence of double parietals in *T. braminus* (Fig. 1, C), and mention that the occurrence of a paired parietal bone "as such escaped the notice of the former investigators, the size of the skull being very small and alizarine preparations of transparency being unknown to them". The claim is baseless, as Jan and Sordelli (1870-1881) figured (Fig. 1, A) the skull of *T. braminus* long before Mookerjee and Das, and showed in it the presence of two separate parietals. Even two years earlier to the publication of Mookerjee and Das's note, Haas (1930) examined the skulls of several species of this genus and came to the conclusion that some species like *T. braminus* (Fig. 1, B) have double parietals throughout their life, while others have a single fused one in the adult condition and separate ones in the young. Haas says :

> "An die Supraoccipitalia oben und die Prootica lateral schließt sich ein großer Knochen an, der fast ganze hintere Schädeldach bedeckt und auch eine starke Erstreckung über die Flanken aufweist : die verwachsenen Parietalia (Fig. 2-4). Die Synostose dieser Knochen tritt erst in späterem Alter auf, wie noch deutliche Spuren der Sagittalnaht bei den kleineren Stücken und den anderen Arten zeigten. Ja, vielleicht kommt es bei den kleinen Arten überhaupt nie zur Synostose, wie bei *T. braminus*. Die verschiedenen Abbildungen, die ich in der Literatur vorfand, zeigten all Übergänge von verwachsenen bis zu paarigen Scheitelbeinen." (p. 21.)

Of the species that have a fused parietal in the adult condition (Fig. 2), mention might be made of *T. diardi* (Boulenger, 1890), *T. lumbricalis* (Boulenger, 1893), *T. bituberculatus* (Haas, 1930) and *T. punctatus* (Haas, 1930), while those having paired parietals (Figs. 1, 3) are *T. braminus* (Jan et Sordelli, 1870-1881 ; Haas, 1930 ; Mookerjee and Das, 1932) and *T. Richardi* (Jan et Sordelli, 1870-1881). It is almost certain that a careful examination of the remaining species of this genus would add to both these types.

The paired, unfused condition of the parietals appears to be a primitive feature valuable for phylogenetic determinations, especially as this character cannot be shown to be an adaptive modification. There is no difference, as far as I have been able to ascertain, in the habits of the different species of *Typhlops*, with which this cranial feature might be associated ; and as Camp (1923) says, " the primitive nature of unfused paired skull elements is usually acceded to in accordance with the fossil evidence." It must, however, be pointed out that " Mehely inclines to the view ....

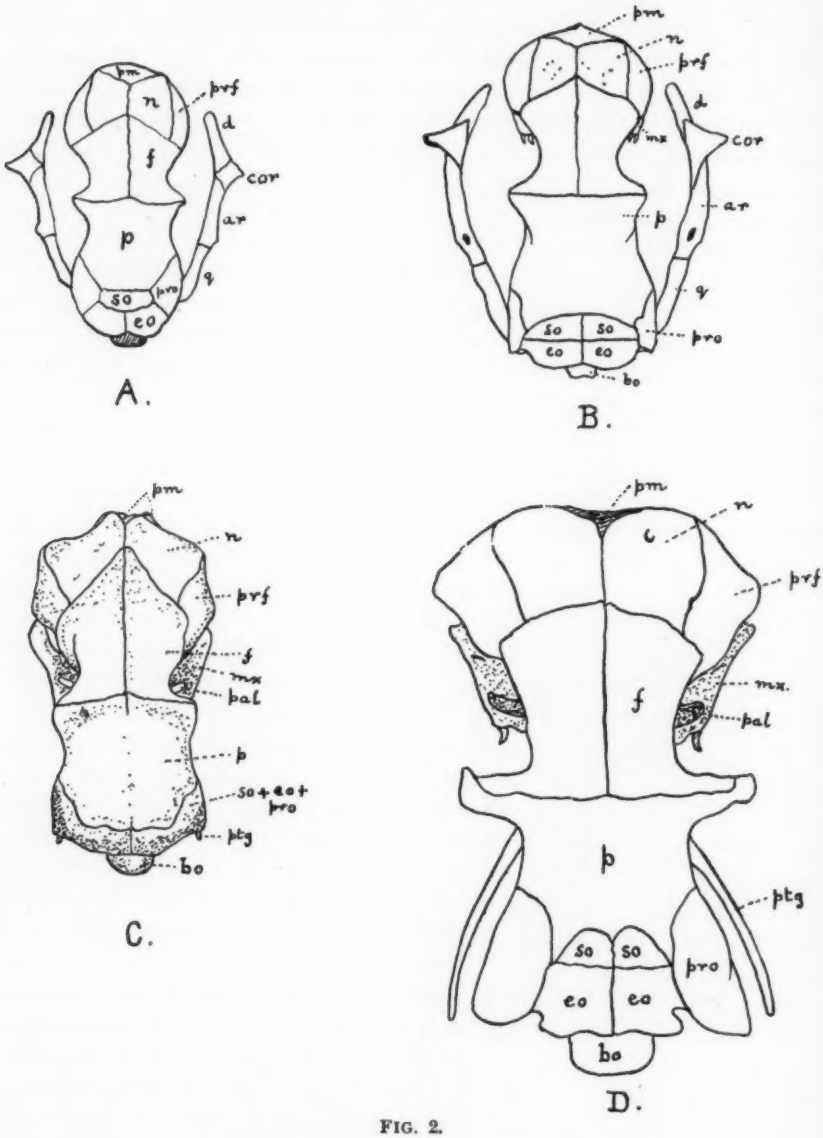


FIG. 2.

The species of *Typhlops* with fused parietals.

A.—T. diardi (Boulenger). *B.—T. lumbricalis* (Boulenger). C.—*T. bituberculatus* (Haas). *D.—T. punctatus* (Haas).

that embryonic and post-embryonic paired conditions may prevail in descendants of a series of ancestors with fused elements, thus upsetting any scheme based on progressive fusion and non-disjunction of once fused elements"; and also that no views on phylogenetic relationships can be regarded as really secure, unless they take not one or two, but numbers of important characters into account.

With regard to both the families *Typhlopidæ* and *Glauconiidæ*, Haas (1930) says, " Beiden Formen fehlt das Supratemporale (Squamosum), das Transversum und das Postfrontale " (p. 87), and none of his figures, accordingly, show any trace of the postfrontals in the skulls (*e.g.*, Fig. 1, B; Fig. 2, C and D) examined by him. The diagrams by Jan et Sordelli (1870-1881), Peters (1882), Boulenger (1890, 1893), and Mookerjee and Das (1932) appear to confirm this notion. In my own preparations, however, the postfrontals are certainly present, being in the form of three or fewer small pieces at the anterior outer borders of the parietals (Fig. 1, D, *pof*). Perhaps these bones should be identified as representing both the postfrontals and the postorbitals, though of our interpretation we cannot be quite sure, especially as the eyes are situated far more anteriorly and do not appear to be related to what apparently corresponds to the orbital region of the other ophidians. I find it difficult to explain how the previous workers missed observing these bony pieces, but I feel that it was perhaps due to the fact that they depended on less reliable methods of skeleton preparation than I have used. As far as I am aware, the method of first staining the bones by alizarin and then clearing them carefully under a dissecting microscope cannot be surpassed for delicacy and accuracy.

According to Boulenger, the families *Typhlopidæ* and *Glauconiidæ* have their " cranial bones solidly united ", and this view is generally believed to be correct. Gadow (1901), Sedgwick (1905) and the other textbook writers appear to believe in its accuracy, and more recently Haas (1930) says, " Der Schädel ist hauptsächlich durch die groβe Festigkeit charakterisiert, eine Anpassung an das Wühlen und Bohren in der Erde.... Die ursprüngliche Beweglichkeit der einzelnen Knochen gegeneinander ist fast vollkommen geschwunden, bloβ der reduzierte Kieferapparat behielt sie, wenn auch in stark abgeänderter Form, bei. Die Schädelkapsel ist ein starres Ganzes, an dem der Kieferapparat bloβ ganz lose suspendiert ist " (pp. 9-10); and further, " All übrigen Knochen bilden ein einheitliches Ganzes."

In spite of these prevalent notions, however, I must draw attention to the wide separation between cranial bones seen in a dorsal view of the skull of *T. braminus* (Fig. 1. D), and point out that the state of affairs as

we find it in this species is hardly what we might expect from the statements of previous workers. The skull bones are certainly not solidly united nor can the cranium be said to be a "starres Ganzes" (rigid whole) or an "einheitlicher Ganzes" (a whole forming one unity). The bones forming the brain-case are separate from each other, and the cleft, running longitudinally in a dorsal view of the skull, is quite characteristic in shape and broadens out cross-wise both at the anterior and the posterior ends of the parietals, the inner sides of which are more or less parallel, and may either curve in a broad sweep away from each other posteriorly (Fig. 1,D) or run straight up to the hind-most edge (Fig. 1, C) of the parietal.

Jan and Sordelli (1870-1881), in their figure of the dorsal view of the skull of this species, show a single fused bone behind the two parietals and regard it as the fused supraoccipitals, exoccipitals and prootics (Fig. 1, A, *so+eo+pro*). Haas (1930) shows two such bones separated from each other by a mesial longitudinal suture and says: "Bei *T. braminus* (weniger ausgesprochen) und *T. bituberculatus* (vollständig), also kleineren Arten, verschmelzen die Prootica mit den Occipitalia lateralia und den Superaoccipitalia zu zwei groβen Knochen jederseits, die einen einheitlichen Ring um das Hinterhauptsloch bilden, der bloβ oben durch eine Sagittalnaht und unten durch das eingeschobene Basioccipitale unterbrochen wird." (p. 18.) Mookerjee and Das (1932) show two bones on either side, but do not label them. In the largest specimen that I have examined, as well as in others, I find that the prootics are separate from the bones of the occipital region and are not fused with them, as Haas and Jan et Sordelli believed. In the skull photomicrographed (Fig. 1, D), for instance, there appear to be two separate bones on the right side, and one on the left, lying to the outer side of the exoccipitals and on the posterior external side of the parietal. None of the specimens I examined—not even the smallest one—shows any trace of the supraoccipitals, and it may very well be that these bones have completely disappeared in this snake. Unless it can be shown that the supraoccipital elements arise in the earlier stages and later on merge with the exoccipitals to form the paired bones on either side of the foramen magnum, it is too much to suppose—as Haas does—that the exoccipitals and the supraoccipitals are fused with each other in *T. braminus*. Perhaps, it would be best to reserve our judgment on this matter, till further evidence accumulates.

4. The Atlas and the Axis.

The first two vertebræ are peculiar in having their elements separate from each other even in the adult condition. A little behind the basisphenoid (Fig. 4), which is produced posteriorly into a minute rounded

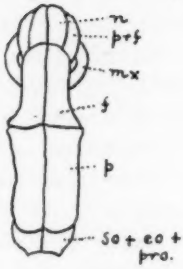
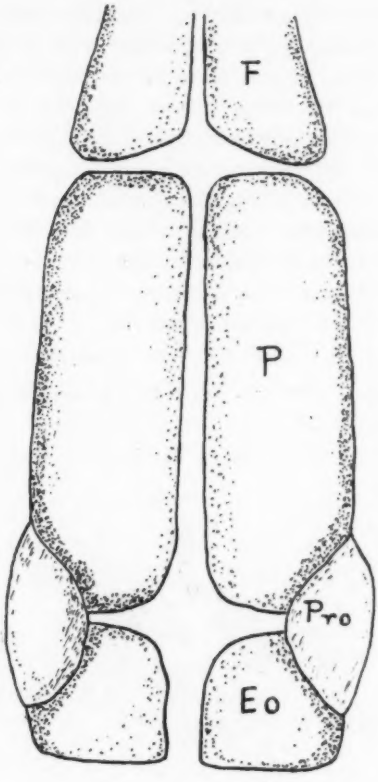


FIG. 3.



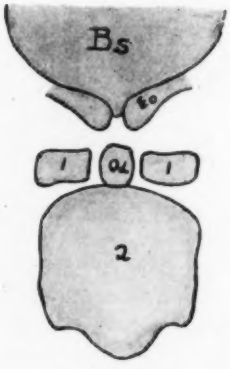


FIG. 4.

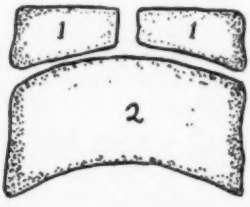


FIG. 5.

FIG. 3. Skull of *T. Richardi*, showing paired unfused parietals (Jan and Sordelli).

FIG. 4. The first two vertebræ from the ventral aspect, with the posterior part of the skull.
Bs, basisphenoid; *eo*, exoccipital; *od*, odontoid; *1*, lateral pieces of atlas; *2*, second vertebra.

FIG. 5. Dorsal aspect of the skull with the first two vertebræ.
Eo, exoccipital; *F*, frontal; *P*, parietal; *Pro*, prootic; *1*, lateral pieces of atlas; *2*, second vertebra.

prominence lying between the two exoccipitals, there is a small rounded piece of bone (*od*), apparently corresponding to the *odontoid* of other squamates, but not fused in this ophidian with the centrum of the second vertebra. On either side of the *odontoid*, two curved lateral bones bound the sides of the neural space, but are separate from each other dorsally (Fig. 5). There is rather a wide gap between the exoccipitals as seen from above and the dorsal aspects of these lateral bones. The second vertebra lacks transverse processes, and has its centrum convex posteriorly to fit in with the anterior concavity of the centrum of the third vertebra. The atlas and the axis of *T. braminus* are apparently primitive in their structure, being yet of unfused elements.

5. *The Other Vertebræ.*

Besides the pre- and the post-zygapophyses, there are in the vertebræ of snakes additional articulations known as *zygosphenes* and *zygantra* (Fig. 6, *z.s*, *z.a.*), which further strengthen the union between the vertebræ

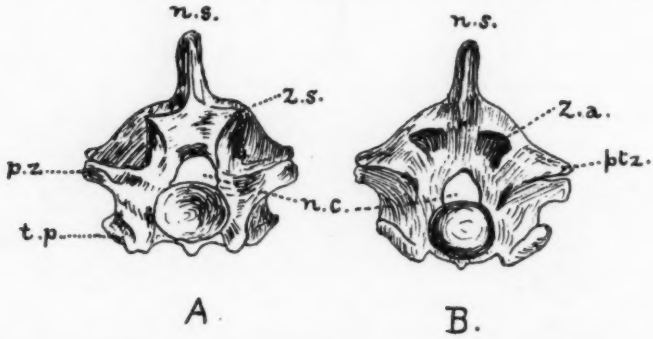


FIG. 6.

Anterior (*A*) and posterior (*B*) view of a trunk vertebra of a Python (afterHuxley). *nc.*, neural space; *n.s.*, neural spine; *pt. z.*, postzygapophysis; *p.z.*, prezy—gapophysis; *t.p.*, transverse process; *z.a.*, zygantrum; *z.s.*, zygosphene.

and are adaptations to allow for the great lateral movements of the body, though restricting vertical flexure. "The zygosphene is a wedge-shaped process at the anterior end of the arch, above and between the zygapophyses, which fits into a corresponding cavity, the zygantrum, at the posterior end of the next preceding vertebra." Williston points out that the zygosphenes and zygantra are "rather, extensions of the zygapophysial articulation about their inner ends", and that the vertebræ of the Ophidia are "always with zygosphenes and zygantra".

Although a great many vertebræ of *T. braminus* were examined for these articulations from various aspects, the zygosphenes and zygantra

appeared at first to be lacking in this ophidian. Further scrutiny, however, showed that they were not actually altogether lacking, but were so rudimentary in their structure that they had eluded the first observations. They (Fig. 7, *z.s.*) are extremely small; the zygantrum is not a cavity or even depression at all, as in other ophidians, but a minute articular surface; and the zygosphene, which is merely a slight conical process, just distinguished at either end of the more or less flattened neural arch between the prezygapophyses, overlaps (rather than fits into) the zygantrum. The zygosphenes and zygantra cannot be said to be dorsal to the zygapophyses, but are at a level with them. Perhaps the condition of these articulations in *T. braminus* is more primitive than in the other Ophidia and gives us a clue as to their mode of origin. Starting as minute overlapping edges of the neural arch internal to the zygapophysial articulations, it appears likely that the zygosphenes and zygantra have evolved into a wedge-and-cavity type of articulation in order to cope with the extra lateral strain on the vertebral column.

The presence and position of the sub-central foramina (Fig. 8) in the vertebræ of *T. braminus* have already been commented upon elsewhere (Mookerjee and Das, 1933, Mahendra, 1935) and need not be discussed here.

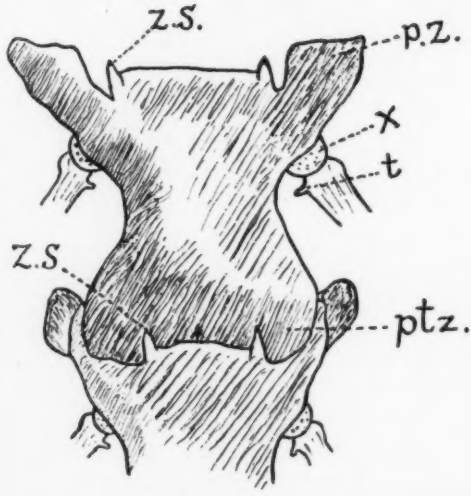


Fig. 7.

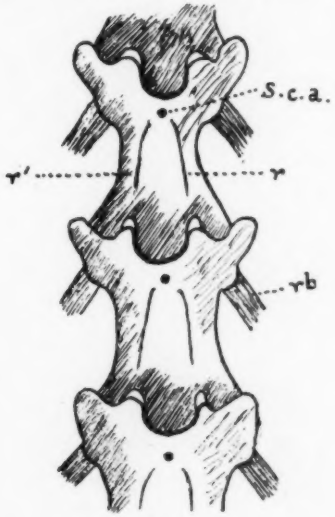


Fig. 8.

Fig. 7. Dorsal aspect of two vertebræ of *Typhlops braminus*.
t, tuberculum; *x*, parapophysis. (Other abbreviations as in previous fig.)

Fig. 8. Vertebral column of *T. braminus* (ventral aspect).
r, *r'*, ridges; *rb*, rib; *s.c.a.*, sub-central aperture.

6. *The Ribs.*

Sedgwick (1905) and several other text-book writers say that in the Ophidia "all the precaudal vertebræ, except the atlas, carry ribs." In *T. braminus*, however, the first *two* vertebræ are devoid of them, while the succeeding precaudal vertebræ possess them. Williston's remark (1925) in this connection, that the first two or three vertebræ in snakes are without ribs, is more accurate. The ribs (Fig. 9) alternate with two scale rows, a

FIG. 9.

Photomicrograph of the body of *T. braminus*, showing the relation between the ribs and the scalation. Each rib extends over two rows of scales.

condition which Stehli (1910) would regard as less primitive than the one, in which a single row of scales corresponds to one body segment. Stehli's conclusions regarding Lacertilian squamation, however, have been controverted by Camp (1923), and as far as the Ophidia is concerned, I feel it advisable to reserve judgment on this point till the end of the present series. Camp says, "Stehli would regard those forms having a single row to each segment as more primitive than those with two rows as he states in his conclusions (p. 795). This would involve the derivation of the normal zunurids from the degenerate Chamæsaura, the normal teiids from the worm-like Bachia, and many other startling cases." (p. 400.)

The ribs are long and slender, and are directed backwards from their vertebral attachment. They are attached to a rounded prominence (*parapophysis*) on the sides of the centra anteriorly near the base of the prezygapophyses; and their distal ends in some cases show a cartilaginous biforked tip. Proximally, a little away from the point of vertebral attachment, the ribs have a small posteriorly directed process (Fig. 10, *t*), which apparently corresponds to the *tubercle* of other forms and is a remarkable feature as the ophidian ribs are generally regarded as having no tubercula, but only capitula.

The caudal vertebræ as is common with burrowing forms, are very few, being only 6 to 8 in number ; and of these only the three or four anterior ones have minute, outwardly-directed ribs, the terminal ones being devoid of them entirely. It is a remarkable fact that two or three vertebræ just ahead, or at, the anal region are distinguished from all the rest by having double ribs on each side (Fig. 11) and they probably correspond to the *sacral*

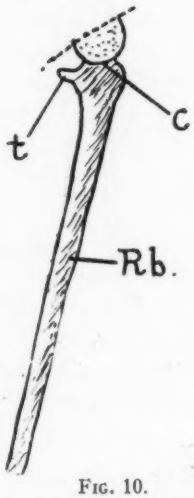


Fig. 10.

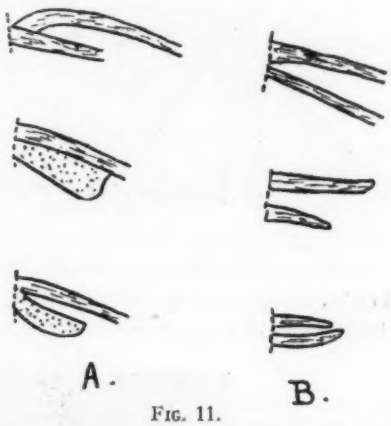


Fig. 11.

Fig. 10. Proximal part of a rib, showing the capitulum (*c*) and the tuberculum (*t*).
Fig. 11. Ribs borne on the three vertebræ of the "sacral" region in two specimens, A and B.

vertebræ of the Lacertilia. In one specimen (Fig. 11, A) two such vertebræ bear a rib along with a plate-like lateral cartilage on each side, and it is not unlikely that the double ribs might really be the vestiges of the ossified anterior and posterior ends of a greatly flattened sacral rib. If this is right, *Typhlops braminus* shows an important primitive feature. In any case the vertebræ at the sacral region are marked off by this feature from the rest, and it would be worth while to find out how far this species shows a condition of fixity in this character, and also whether other species of *Typhlops* show a correspondence to *T. braminus* in this region.

7. *Pelvic Vestiges.*

Peters (1882), in his diagram of *Typhlops dinga*, shows two small bones just anterior to the cloacal aperture and regards them as the vestiges of a pelvic girdle. Boulenger (1890), in his description of the family, says, "Vestiges of pelvis, reduced to a single bone on each side". Essex (1927) examines several specimens of 11 species of *Typhlops* and finds in many of

them, besides the two bones, which he regards as ischia, vestiges also of ilia, pubes and hypo-ischia in the form of cartilaginous structures, which vary a great deal in their relative proportions and development in the various species. He thinks that the girdle as found in *T. delalandii* (Fig. 12) is the most primitive, and that the one in *T. braminus* is extremely degenerate. In the latter case (Fig. 13), according to him, it consists of "merely two very thin ischia with small cartilages at either end".

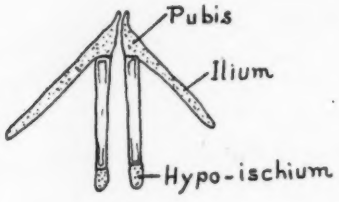


FIG. 12.

FIG. 13.

FIG. 12. Pelvic girdle of *Typhlops delalandii* from Eastern Province, S.A. (after Essex).
FIG. 13. Pelvic girdle of *Typhlops braminus* from Godavery Valley, India (after Essex).

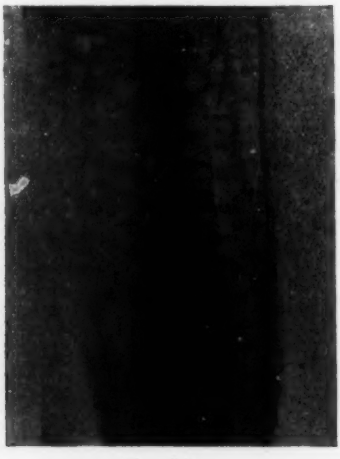


FIG. 14.

FIG. 14. Photomicrograph of the pelvic vestiges in *T. braminus* from Agra.

In the specimens that I have examined I have not been able to distinguish the cartilages (supposing that they *are* present) apart from the slender osseous rods which lie just anterior to the cloacal aperture. I have not only

examined dissected specimens, but also *whole* specimens which have been stained by alizarin and cleared before microscopical examination, and so I am of the opinion that there is a great deal of variation evinced by the species in the feature of its pelvic vestiges and that the cartilages, which correspond to pubis and hypo-ischium according to Essex, may or may not be present.

The bones, representing the pelvic vestiges (Fig. 14), are delicate and slender, slightly more than 1 mm. long and tapering a little from behind forwards, besides converging to some extent towards each other. They lie almost free just below the skin, and in one specimen at least one of these bones shows a slight prominence at its inner side towards the posterior end.

8. Summary.

A careful study of the osteological features of four alizarin-stained specimens of *Typhlops braminus* shows that:

1. The parietals are paired: a feature which is exceptional in the Ophidia. In this connection, the relevant literature is reviewed, the parietals as figured by other authors in various other species of *Typhlops* are mentioned, and the suggestion that the presence of unfused parietals might be a primitive feature is considered.

2. The postfrontals (and possibly, the postorbitals) are present.

3. The cranial bones cannot be said to be solidly united, as Boulenger supposed. The median cleft in a dorsal view of the skull is characteristic in shape.

4. The supraoccipitals are entirely absent.

5. The odontoid lies between the two lateral pieces of the atlas and is separate from the second vertebra.

6. The zygosphenes and zygantra are extremely small and primitive in structure.

7. The ribs alternate with two scale rows, have vestiges of the tubercula, and are distinguished particularly in the sacral region.

8. The pelvic vestiges are in the form of two rod-shaped bones anterior to the cloacal aperture.

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STUDIES ON THE MECHANISM OF BIOLOGICAL NITROGEN FIXATION.

Part I. Economy of Carbon during Fixation of Nitrogen by the Mixed Flora of the Soil.

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Received December 20, 1935.

In view of its profound scientific interest and very great practical importance, biological fixation of atmospheric nitrogen has attracted considerable amount of attention during recent years. In addition to numerous studies on soil or seed inoculation, special attention has also been paid to the physiology of the organisms concerned and the various factors influencing fixation. Much interest has centred round the mechanism of fixation, especially on the nitrogen side and the energy changes attendant thereon. Valuable work has also been done on the related carbon transformations, especially by Stoklasa (1908), Bonazzi (1921) and Ranganathan and Norris (1927), but further information is needed on certain important aspects of the problem. Thus, it is not definitely known as to how the different forms of organic matter are utilised in the fixation ; at what particular stage of the decomposition the fixation begins ; what portion of the degraded organic matter is taken up by the organisms and as to how far the growth of the organisms is related to the fixation. With a view to throwing some light on these and related aspects of the problem, the present series of investigations were undertaken.

Experimental.

Technique.—In the study of carbon transformations, it would be desirable to separate living organisms from other carbonaceous substances present in the medium. Unfortunately, this is not possible because the presence of calcium carbonate is essential for the fixation. The carbonate always comes down with the organisms and any method of separating them will be only partially successful. The analytical procedure was therefore modified to include carbonates and may be described as follows :—At stated intervals, samples of suspension containing the mixed flora of the soil were taken out and analysed for their carbonate, organic carbon and total nitrogen

contents (Subrahmanyan, Narayanayya and Bhagvat, 1934; A.O.A.C., 1930). Parallel samples were centrifuged and the suspended matter, together with the insolubles, separated from the clear supernatant. They were then analysed for their organic carbon and nitrogen contents. In a further sample, the residual sugar, if any, was estimated (Bhaskaran *et al.*, 1934). From these results, the distribution of carbon was calculated as follows :—The difference between the total carbon originally taken and that left behind at

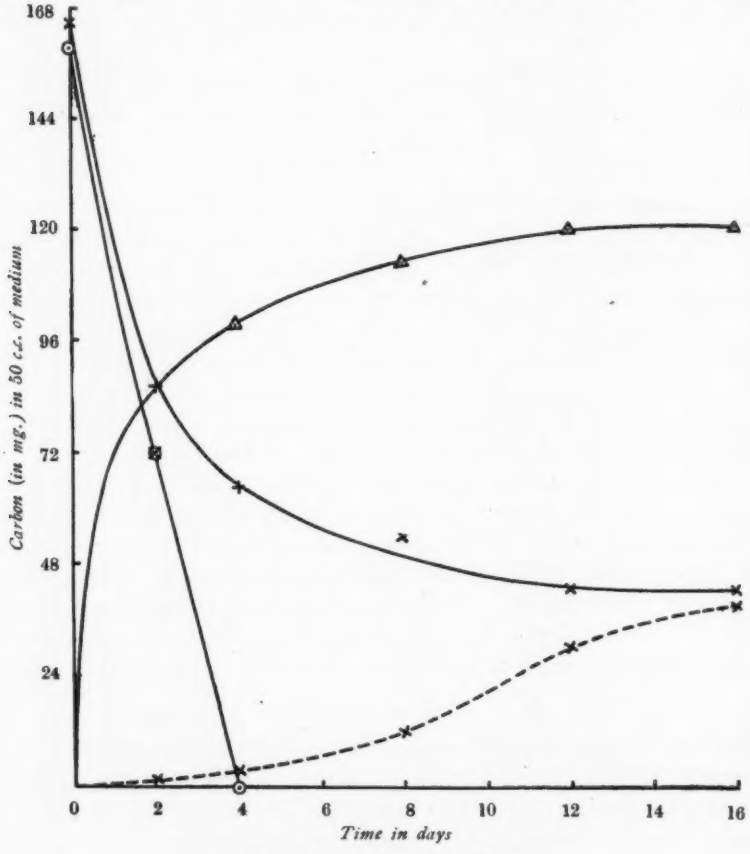

FIG. 1. Distribution of Carbon

Carbon (Total) present in the system
" present in the sediment
" lost as gas
" present as sugar

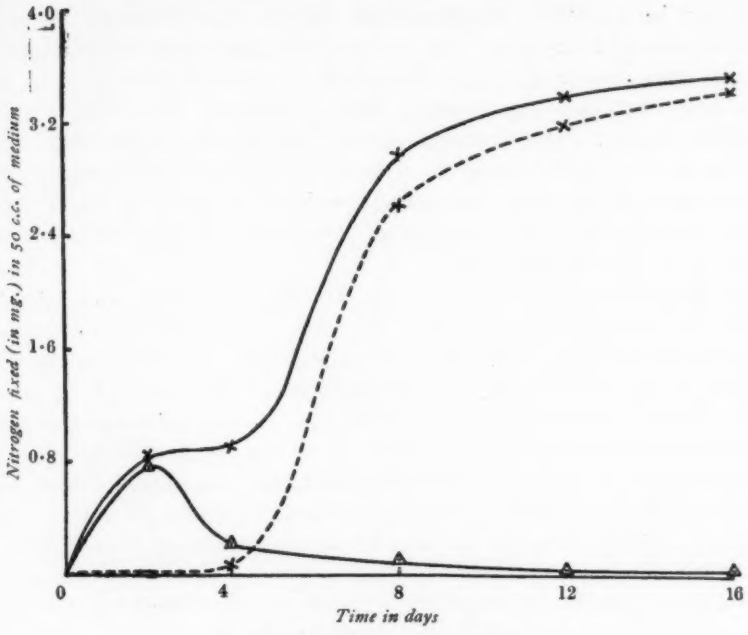

FIG. 2. Distribution of Nitrogen

each stage gave an estimate of the quantity lost as gas. The difference between total carbon in the sediment and the carbonate present in it yielded the figure for organic carbon in the organism. The difference between the total organic carbon of the supernatant and that actually present as sugar represented the soluble products formed by fermentation. The separate estimations of nitrogen helped to differentiate between that present in the organism and that occurring in soluble form in the supernatant.

In nitrogen fixation studies, mannitol is generally used as the source of carbon, but as there is no accurate method for estimating that hexitol, glucose was used in its place. Otherwise, the medium was similar to that originally proposed by Ashby (1907). Since, under natural conditions, the mixed microflora—rather than any single organism—are concerned with the various transformations, soil itself (0·1 g. of air dried material for 50 c.c. of medium) was used for inoculation. The incubations were at 30° and the results have been presented in Figs. 1 and 2.

It may be seen from the above that all the sugar was used up in the course of the first four days. The corresponding loss of nitrogen in gaseous form was also considerable. The subsequent changes, though slow, were highly significant from the point of view of fixation. The organic matter in solution was steadily converted into insoluble forms representing mostly the tissue material of the organism. There was also marked fixation of nitrogen, especially between the fourth and the eighth days. The major part of the fixed nitrogen was present in soluble form but a small part was also present throughout in soluble condition.

Microscopic examination of the supernatant obtained after centrifuging, especially between the fourth and the eighth days, showed that it was not completely free from living cells. Moreover, it was not clear whether the acids, which were the chief products of fermentation, would account for all the organic matter in the supernatant. A fresh series of experiments was, therefore, carried out, first adding a flocculent to precipitate the organism and then studying the distribution of carbon and nitrogen. A number of flocculents, Kieselguhr, calcium sulphate, freshly precipitated barium sulphate, copper hydroxide, dialysed iron and alumina cream, (preparation C of Willstätter and Kraut, 1923) were tried, but the last was found to be most effective in precipitating the organisms, especially in the early stages, when it is so difficult to separate out the young cells. In addition to the above, the quantities of organic acids present at different stages were determined. Lactic acid was estimated according to Subrahmanyan (1929), the total volatile fatty acids by the method of Birkinshaw and Raistrick (1931) and the distribution according to Virtanen and Pulkki (1928). The results have been presented in Tables I to III.

TABLE I.

Distribution of carbon (suspension treated with alumina cream and then centrifuged).

Time in days	Organic carbon (in mg.) in 50 c.c. of medium				Lost as gas (as mg. of carbon)
	Total	Present as sugar	Present in sediment*	Present in supernatant	
0	173·7	170·4	..	..	..
2	111·7	83·6	13·6	94·8	62·0
4	55·0	Nil	24·2	27·5	118·0
8	53·4	..	39·8	10·3	120·3
12	52·5	..	39·9	9·3	121·2
16	50·5	..	39·4	7·8	123·2

* The soil used for inoculation contained 3·3 mg. of carbon, but correction has been applied for this.

TABLE II.

Production and distribution of organic acids.

Time in days	Organic acids (as mg. of C) in 50 c.c. of medium					
	Non-volatile	Volatile				Total
	Lactic	Acetic	Propionic	Butyric	Total	
2	6·1	2·7	0·6	3·8	7·1	13·2
4	12·8	3·1	0·4	3·5	7·0	19·8
8	Nil	..	..	..	5·8	5·8
12	Nil	..	..	..	4·8	4·8

TABLE III.

Distribution of nitrogen.

Time in days	Nitrogen fixed (as mg.) in 50 c.c. of medium			C—N ratio of mucilage
	Total Nitrogen	Nitrogen in sediment	Nitrogen in supernatant	
2	0·78	0·39	0·39	43·9
4	1·26	0·39	0·87	62·1
8	2·42	1·93	0·49	20·6
12	3·07	2·90	0·17	13·8
16	3·15	2·95	0·20	13·4

The above observations show that a part of the carbon in the sediment was derived from the sugar itself while the rest originated from the immediate products of decomposition. The major part of the carbon in the supernatant was accounted by the organic acids. The latter diminished in quantity after the 8th day and were presumably taken up by the organisms.

Working with *Azotobacter chroococcum*, Ranganathan and Norris (*loc. cit.*) observed that the decomposition of sugar was comparatively slow; that the fixation of nitrogen was complete before the entire quantity was used

up. They record, however, that there was a sudden decrease in volatile acids between the 9th and the 13th days. Regarding the first observation, it is difficult to determine whether it was due to the slow action of *Azotobacter* or the smallness of the inoculum. As the sugar was present all through in the medium, it is not possible to ascertain whether any of the products of fermentation was used up in the fixation. The disappearance of a portion of the volatile acids is, nevertheless, significant and lends indirect support to our observation.

The C-N ratio of the sediment narrowed rapidly between the 4th and the 8th days and attained a steady value by the 16th day.

Starting with the products left on the 4th day, it would appear that the return of nitrogen for the carbon utilised was favourable. When reckoned, however, on the basis of the original sugar, it would be found to be very small (1 : 55).

Fixation of Nitrogen by the residue after decomposition of sugar:— The fermented suspension (4th day) was filtered through Kieselguhr and the clear filtrate subjected to discontinuous steam sterilization. It was then inoculated with the soil and the extent of fixation at the end of 4 and 8 days compared with that in unsterilised control. (The quantity of inoculum was increased to 1 g. so as to correspond to the growth during the first four days. The necessary correction for added nitrogen was made when calculating the quantities actually fixed.)

In another series, the supernatant was separated by centrifuging (4000 r.p.m. ; 15 min.) and inoculated with soil. To the sediment sterile water was added to make up the volume and the suspension incubated as before.

The quantities of nitrogen fixed in the two sets of experiments have been presented in Tables IV and V.

TABLE IV.

Time in days	Nitrogen fixed (in mg.) by 50 c.c. of medium	
	Filtrate sterilised and freshly inoculated	Unsterilised (control)
4	0·25	2·10
8	1·62	2·62

TABLE V.

Time in days	Nitrogen fixed (in mg.) in 50 c.c. of medium		
	Supernatant	Sediment	Unsterilised (control)
4	1·25	0·39	2·10
8	1·62	0·44	2·62
12	1·67	0·44	2·77

The above observations afford direct proof to show that the residue after decomposition of sugar can fix nitrogen. The supernatant is the more potent fraction, while the sediment (containing the bulk of the organisms), when left by itself, does not fix any appreciable quantity.

Discussion.

The results of the present enquiry are of much practical interest. In the first place, they show that in presence of the mixed flora—which represent the natural conditions of the soil—the major part (about 70 per cent.) of the added sugar is wasted. The return of nitrogen for the carbon spent is very small. On the other hand, the residual organic matter, consisting of the acids and other products of fermentation, is more efficiently utilised not only for the growth of the organisms but also for the fixation of nitrogen.

It is well known that nitrogen fixers can use organic acids (or their salts) as sources of energy. Since in presence of the soil, sugars are so easily decomposed, it would appear to be desirable to convert them into more stable products before application to the field.

Our researches have already shown that, under conditions of restricted air supply, gas production is greatly reduced. The major part of the sugar is converted into organic acids, chiefly volatile fatty acids (Bhaskaran, private communication). If these could be subsequently applied to the field in the form of their mixed calcium salts, then there would be greater fixation of nitrogen than would otherwise be possible.

In recent years, much interest has centred round the possibility of utilising cane molasses as fertiliser. The mechanism of the fertilising action is still not fully understood, but the recent observations of Dhar and Mukherji (1934, 1935) would suggest that it is at least partly due to the fixation of atmospheric nitrogen. On the other hand, it has been shown by a number of previous workers that molasses rapidly decomposes in the soil so that the

return of nitrogen for the carbon utilised would be very small. The procedure described above would appear to provide a convenient means of applying molasses to land and, at the same time, obtaining greater fixation of nitrogen.

Further researches on the foregoing and allied aspects of the problem are in progress and will be described in subsequent communications.

Summary.

During decomposition of glucose by the mixed flora of the soil, a considerable part (over two-thirds) of the sugar is converted into gaseous forms in the course of the first four days. The residue is present in water-soluble forms, chiefly as organic acids. The latter are largely utilised for the fixation of nitrogen.

The water-soluble products left after the decomposition of sugar, if inoculated with the mixed flora of the soil, can fix nearly two-thirds as much nitrogen as the original sugar itself. The solid sediment (including the major part of the organism), when left by itself, does not fix any appreciable quantity of nitrogen.

The authors' thanks are due to Mr. M. Sreenivasaya for his keen interest and much valuable co-operation in the earlier stages of the research.

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STUDIES ON THE MECHANISM OF BIOLOGICAL NITROGEN FIXATION.

Part II. Rôle of Lime in the Fixation of Nitrogen by the Mixed Flora of the Soil.

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Received December 20, 1935.

(Communicated by Prof. V. Subrahmanyan, D.Sc., F.I.C.)

The rôle of calcium in the fixation of nitrogen by *Azotobacter chroococcum* has been studied by Burk and Lineweaver (1931), Schrörder (1932) and others. They have adduced evidence to show that calcium is essential to the fixation and cannot be replaced by any other element with the exception of strontium.

In the course of our studies on the carbon transformations attendant on fixation of nitrogen by the mixed flora of the soil, it was found that calcium carbonate could not be replaced by many of the soluble salts. Moreover, the quantity of carbonate required for efficient fixation was fairly large, so that the beneficial effect could not be attributed exclusively to the calcium ion. The position required further elucidation, so the present enquiry was undertaken.

Experimental.

It is now generally recognised that the mixed microflora of the soil decompose sugars to form acids. Since acidity is inimical to fixation (Yanagata and Itano, 1923; Christensen, 1927; Burk *et al.*, 1934) some experiments were first carried out substituting phosphate buffer of different concentrations for calcium carbonate in the usual Ashby's medium. The mixed flora were inoculated in the form of air-dry soil (red sandy loam) into 100 c.c. lots of the medium. The cultures were incubated at 30°. Samples were taken out at short intervals and examined for changes in H-ion concentration and extent of nitrogen fixation. The former was determined colorimetrically, and the latter according to Kjeldahl method (Tables I and II).

It was observed that there was practically no growth of organism when the concentration of phosphate was high. At the lower concentrations there was some growth. But the medium became steadily acid. The quantities of nitrogen fixed were less than half those observed in the normal medium. As more or less similar results were obtained in the case of controls (without

TABLE I.

Effect of substituting phosphate buffer for calcium carbonate (in Ashby's medium) on fixation of nitrogen by the mixed flora of the soil.

Medium (phosphate buffer conc. in the medium)	Nitrogen fixed (in mg.) at the end of (time in days)			
	3	6	12	25
M/15	0·39	0·73	0·91	0·76
M/30	0·55	0·87	1·68	1·65
M/60	0·63	0·31	3·09	3·04
M/120	0·69	0·73	3·42	3·06
Without buffer or $CaCO_3$	Nil	1·98	3·52	4·38
Ashby's (control) ..	0·90	1·81	8·53	8·90

TABLE II.

Changes in P_H consequent on substituting phosphate buffer for calcium carbonate in Ashby's medium.

Phosphate buffer concentration in the medium	H-ion concentration (as P_H) at the end of (time in days)			
	3	6	12	25
M/15	7·2	7·2	7·2	7·2
M/30	7·2	7·2	6·9	6·6
M/60	7·3	7·2	5·1	4·6
M/120	7·3	7·2	5·1	4·5
Without buffer or $CaCO_3$	6·8	5·1	4·7	4·4
Ashby's (control) ..	7·0	6·8	7·0	7·2

either phosphate or calcium carbonate), it may be inferred that addition of moderate quantities of phosphate buffer made practically no difference to fixation of nitrogen. On the other hand, excess of phosphate discouraged the growth of organisms and although the H-ion concentration remained constant, there was practically no fixation.

Effect of adjusting the reaction at short intervals.—The foregoing observations, while emphasising the importance of reaction, did not show clearly whether calcium was required in preference to other kations. With a view to throwing some further light on this aspect of the problem, the medium (prepared without calcium carbonate and inoculated with the mixed flora) was neutralised with caustic soda, potash or lime at daily intervals, the P_H being adjusted to 7·2 in each case. Samples were taken out once in 4 days and examined not only for the change in P_H during the preceding 24 hours, but also the total nitrogen fixed upto the time of observation. The results have been given in Tables III and IV.

TABLE III.

Reaction adjusted with	Nitrogen fixed in mg. at the end of (time in days)		
	4	8	12
KOH..	1·33	2·63	2·63
NaOH	1·30	3·13	2·68
$Ca(OH)_2$	1·53	2·93	3·63
$CaCO_3$ (Ashby, control)	2·35	7·75	8·15

TABLE IV.

Reaction adjusted with	P_H change during preceding 24 hours—Observations made at the end of (time in days)			
	0	4	8	12
KOH	7·5	7·0	7·1	6·8
NaOH	,,	6·0	6·0	6·6
$Ca(OH)_2$	,,	6·3	6·0	6·0
$CaCO_3$ (Ashby's medium-control) ..	,,	7·4	7·5	7·4

It may be seen from the above that the changes in reaction were fairly rapid and could not be controlled, at any rate, by either caustic soda or lime. The quantities of nitrogen fixed were also comparatively small. Neutralisation with lime yielded slightly better results, but even they were small

as compared with the control (with calcium carbonate). These observations show that the increasing fixation consequent on the addition of calcium carbonate is not so much due to the supply of calcium as to the steady neutralisation of the acid products of reaction by the excess of calcium carbonate already present in the medium. On the other hand, if the reaction is adjusted from day to day, the medium turns so rapidly acid that fixation of nitrogen is seriously impaired.

Substitution of calcium carbonate by other carbonates.—With a view to determining whether other insoluble carbonates can take the place of calcium carbonate, the following experiment was carried out :—To one set of flasks containing Ashby's medium without calcium either as sulphate or carbonate, 1 g. each of magnesium, strontium or barium carbonate was added. To another set containing medium of similar composition, calcium sulphate was added at the rate of 0·01 g. to 100 c.c. of medium, together with 0·5 g. of one of the abovementioned carbonates. The media were then inoculated with the mixed flora of the soil and the quantities of nitrogen fixed at different stages determined as before. The results have been presented in Tables V and VI.

TABLE V.

Effect of substituting calcium carbonate by other carbonates. Ashby's medium without any calcium.

Carbonate added	Nitrogen fixed in mg. at the end of (time in days)		
	4	8	12
$MgCO_3$	0·44	0·74	2·08
$BaCO_3$	0·49	2·05	3·28
$SrCO_3$	3·30	6·80	6·89
$CaCO_3$ (control) ..	2·10	7·14	8·40

It may be seen that there was slight increase in fixation consequent on addition of minute quantities of calcium sulphate. The improvement is not, however, so considerable as to suggest that calcium ion supplied in such small concentrations is the chief factor controlling fixation. The results obtained with strontium carbonate do indeed show that calcium carbonate can be replaced by it. These observations are in agreement with those of Horner and Burk (1934).

TABLE VI.

Effect of substituting calcium carbonate by other carbonates. Ashby's medium with minute quantities of calcium sulphate (0·01 g. to 100 c.c. of medium).

Carbonate added	Nitrogen fixed in mg. at the end of (time in days)		
	4	8	12
$MgCO_3$	1·41	2·29	4·06
$BaCO_3$	1·53	3·16	4·48
$SrCO_3$	3·38	7·09	7·69
$CaCO_3$ (control) ..	2·90	7·40	8·10

It is difficult to explain why magnesium and barium carbonates did not yield as high results as calcium or strontium carbonate. It is probable that the high concentrations of the corresponding metallic ions (which steadily pass into solution) are not helpful to the growth or active functioning of the nitrogen fixers. Further study on this aspect of the problem is needed before any definite conclusion can be drawn.

Summary.

The rôle of lime in the fixation of atmospheric nitrogen by the mixed flora of the soil has been studied.

It has been found that consequent on the decomposition of sugar, there is much production of acid that neither a dilute solution of buffer nor periodical addition of alkali helps to maintain the medium at the optimum reaction. In presence of excess calcium carbonate, the acids are neutralised as fast as they are produced with the result that the medium continues to remain neutral and the fixation proceeds unimpaired.

Strontium carbonate can replace the calcium salt in the nitrogen fixing medium. Barium or magnesium carbonate is not so useful. Addition of minute quantities of calcium sulphate helps to improve the fixation.

The author's thanks are due to Prof. V. Subrahmanyan and Mr. M. Sreenivasaya for their kind interest in the progress of the work and helpful criticism.

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ON THE AGE OF THE DECCAN TRAPS NEAR RAJAHMUNDRY.

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Introduction.

From very early times in the study of Indian geology the problem of the age of the Deccan traps has been the subject of some difference of opinion, and the different views expressed from time to time on this interesting question may be classified as follows :—

(*a*) the entire Deccan traps are early tertiary in age (eocene) ;
(*b*) they wholly belong to the upper cretaceous ; and
(*c*) they were poured out in the epoch which covers the close of the mesozoic and the early beginnings of the tertiary era.

Our interest in the study of this problem has been revived by a recent paper on "The Deccan Traps : Are they Cretaceous or Tertiary ?" by Dr. Sahni.[1] In this very valuable communication Dr. Sahni has drawn pointed attention to the considerable body of direct palæo-botanical evidence he has recently gathered from the inter-trappean beds of the Nagpur-Chhindwara region in support of assigning an eocene age to the Deccan traps as a whole —thus going back to an opinion which, as Dr. Sahni has pointed out, was the earliest to be put forward by such pioneer geologists as Malcolmson, Hislop and Hunter, and T. Oldham.

In the course of a reply to Dr. Sahni's paper, Dr. C. S. Fox[2] has discussed the whole problem and placed before us the other point of view, as it were, of this question. One important fact which emerges from Dr. Fox's communication is that though the official opinion of the Geological Survey of India that the Deccan traps are wholly upper cretaceous in age has not been publicly revised for want of sufficient evidence, there is no doubt that the view "that the Deccan trap series is entirely of cretaceous age is largely out of date and is not the view of those officers (of the G. S. I.) who

[1] *Curr. Sci.*, Oct. 1934, 3, No. 4, 134-136.
[2] *Curr. Sci.*, March 1935, 3, No. 9, 428-430.

are most familiar with these lavas." In the light of more recent studies, Dr. Fox and his colleagues with whom he has discussed this problem are now prepared to accept Dr. W. T. Blanford's view that this volcanic period (the Deccan traps) possibly straddles the close of the cretaceous and the beginning of the tertiary era.

To any one interested in this problem of the age of the Deccan traps, these two papers mentioned above are of very great value, since they review the whole position up to date and enable us to appreciate further discussion. In the course of their remarks both Dr. Sahni and Dr. Fox have referred to the Deccan traps and their associated sediments found near Rajahmundry, and it has generally been recognised that studies in this area are likely to be very useful in determining the age of these lava flows. As Dr. W.T. Blanford wrote so far back as 1879,[3] "the most important clue to the correlation of the volcanic rocks with the known series of fossiliferous deposits might be expected to be obtained from the marine beds associated with the volcanic formations at Rajahmundry."

We have recently examined the fossiliferous sediments associated with the Deccan traps near Rajahmundry and the main object of the present paper is to briefly review the geology of this area, with special reference to certain new palæontological observations made by us and discuss their bearing on the age of these lava flows.

Rajahmundry area.

The infra-trappean beds.—It is well known that near Pungadi, Gowripatam and Dudukur there occurs below the lowest trap flows a gray sandy fossiliferous limestone of marine origin; and between this infra-trappean limestone and the overlying trap there is an unconformity. This limestone and the included fossils have been described by King[4] and he comes to the conclusion that " the majority of fossils are such as are usually considered as of tertiary age" ; and according to W.T. Blanford[5] " Although the whole facies is tertiary, there is a remarkable absence of characteristic genera, and the chief distinction from the cretaceous fauna of the upper beds in South India is simply the want of any marked cretaceous form. The fauna is distinctly marine. .....The balance of evidence is rather in favour of referring the latter (the Pungadi infra-trappeans) to cretaceous times rather than to tertiary. They may be of intermediate age."—an opinion very

[3] *A Manual of the Geology of India*, 1879, Part I, 329.

[4] *Mem. Geo. Sur. Ind.*, 1880, **16**, Article 3, 42.

[5] *A Manual of the Geology of India*, Part I, 316.

similar to that expressed by H. F. Blanford[6] in describing the age of the Niniyur beds in the Trichinopoly District when he says: "There is much resemblance between some of the species (of the Niniyur fossils) and those of the Rajahmundry beds, the fossils of which have been described by the Rev. S. Hislop, and one species of *Turritella* appears to be identical with *T. prælonga* of that formation. ....It will be seen from the above that the tertiary aspect of the fossils of the Niniyur bed is more due to the absence of characteristic cretaceous forms than to the presence of those which we have been accustomed to think as peculiar to tertiary deposits; but the latter are not entirely wanting."

In this connection we may point out that sometime back the late Prof. H. C. Das-Gupta[7] collected from this very infra-trappean bed near Dudukur *Venericardia* (*Cardita*) *Beaumonti*, d'Arch.—a fossil of great stratigraphical value in Indian geology. On this and other palæontological evidences Prof. Das-Gupta also concludes that "the Dudukur fossiliferous band should be placed under the upper part of the upper cretaceous age" and thus must occupy a very high position in the upper cretaceous system. It is also well known that in the Niniyur beds of the Trichinopoly area we get *C. Jaquinoti*, which is believed to be identical with *C. Beaumonti*, and we know, on other evidences, that the Niniyur stage marks almost the passage from the uppermost cretaceous to the lower tertiary. Thus it is obvious from all considerations that this infra-trappean limestone in the Rajahmundry area is at least uppermost cretaceous in age; and it is on this bed that the lowest trap flows of this area unconformably overlie. Now the question is, what exactly is the age of these traps? To understand this we must now focus our attention on the inter-trappean beds.

The inter-trappean beds.—These have been elaborately described by King from exposures noticed near Kateru, Rajahmundry, Gowripatam and Dudukur, together with the more common fossils found in them. After examining the evidence afforded by these fossils King[8] writes as follows regarding the age of these beds: "The inter-trappean beds are clearly of the trappean period and they are in some respects rangeable with Hislop's inter-trappeans of Central India; but the latter are essentially of lacustrine or fresh-water origin, while the Pungadi and Kateru fauna is estuarine. However, there are three shells common to the two, *viz.*, *Paludina normalis*, *Physa prinsepi*, and *Lymnea subulata*, and as it appeared when Hislop wrote there

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[6] *Mem. Geo. Sur. Ind.*, 1865, **4**, Article I, 141.

[7] *Qua. Jou. Geo. Min. Met. Soc. Ind.*, June 1933, **5**, No. 2, 59-66.

[8] *Loc. cit. supra*, page 53.

seemed very good reason for his conclusion that the rocks of the one locality are a fair estuarine representative of the lacustrine rocks of the other, and that they are of lower eocene age. I am unable myself to enlarge on or narrow Hislop's generalisation further than to instance the fact that *the traps do not appear to be dissociated from the infra-trappean beds to such an extent of unconformity as the supposedly upper eocene age of the inter-trappean beds would require.*" On the question of the age of these inter-traps in the Rajahmundry area, Dr. W. T. Blanford[9] says : "The most marked feature of this fauna is its distinctly estuarine character. .... The inter-trappean beds of Rajahmundry were deposited in brackish water which was supplied with fresh-water by streams, but which was also in communication with the sea. .... On the whole it may be safely asserted *that no tertiary alliances of any value have been detected amongst the inter-trappean Rajahmundry fossils, and that their relations are rather with the upper cretaceous rocks of Southern India, although the connection is not strong.*" (Italics in these two quotations are ours.)

From the above it will be obvious that while both King and Blanford have not straightaway accepted Hislop's view of the lower eocene age of these inter-traps, they are not prepared to oppose this idea as improbable, nor have they said definitely that these beds are only upper cretaceous. Their attitude in this matter is very clear. King's hesitation to readily accept Hislop's view is merely due to his doubting whether the unconformity between the infra- and inter-traps in this area is of sufficient magnitude. To W. T. Blanford the view that the inter-trappeans are of upper cretaceous age is more acceptable, not because there is any strong connection with the upper cretaceous rocks of Southern India but because 'no tertiary alliances of any value have been detected among the inter-trappean Rajahmundry fossils.' In this connection we should like to refer to some interesting palæontological finds made by us in the course of a recent detailed examination of some of the inter-trappean beds of this area.

Kateru inter-traps.

One of the best exposures of the inter-traps near Kateru is seen in a small quarry about 500 yards to the north of the sanatorium rise and about 200 yards to the west of the Koraikonda road. Forming part of the inter-trappean bed here we have a thin band of a dirty green marl which on washing has yielded the remains of numerous *Chara* fruits. A detailed account of these will be separately published and we might only mention here that the following species of *Chara* are well represented: *C. Wrightii* Salter, *C. helicteres*

[9] *Loc. cit. supra*, pages 318-319.

Brong., *C. cælata* Reid and Groves, *C. turbinata* Reid and Groves, *C. vasiformis* Reid and Groves, and *C. strobilocarpa*, Reid and Groves.

The value of Charophytic remains in the correlation of estuarine and fresh-water deposits lying in isolated basins is well known. As Reid and Groves[10] observe : "In the correlation of far distant deposits we need a group of fossils easily dispersed and therefore widely spread, having many generic and specific forms of limited range in time, with specimens occurring in profusion in a determinable state ; in short, for zonal work in fresh-water strata we require a group somewhat equivalent to the Graptolites in wide distribution and characteristic forms. One group (the Charophyta) seems to possess all these characteristics, if we can obtain sufficient material." A reference to the stratigraphical distribution of these *Chara* species mentioned above from the inter-traps of Kateru shows that they are all distinctive early tertiary types—especially the three most common forms *C. Wrightii*, *C. helicteres*, and *C. cælata*—and thus serve to indicate the age of this inter-trappean bed.

In this connection it would be interesting to recall that some fossil *Chara* have been recognised long ago from the lower inter-trappean beds near Nagpur and one species has been described under the name *C. Malcolmsoni*. We have so far not noticed this form in the Rajahmundry material.

Pungadi-Dudukur inter-traps.

Thin sections of the limestones forming the lowest beds of the inter-trappean series near Pungadi and Dudukur have revealed among other fossils remains of algæ belonging to the family Dasycladaceæ. These algæ are being studied in collaboration with Dr. Julius Pia (of the Natural History Museum, Vienna) but even a preliminary examination of this material has revealed the occurrence of the important form, *Acicularia* which is generally recognised as a distinctive early tertiary type. Here then we have another evidence on the age of these inter-trappean beds pointing in the same direction as the *Chara* of the Kateru inter-traps. Thus it would appear that the position in the Rajahmundry area is merely this ; we have the infra-traps of at least upper cretaceous age, and overlying this unconformably we have the trap flows whose age as determined by the fossils in the inter-trappean beds is at least lower eocene.

Before proceeding further we should like to say a few words regarding the exact mode of origin of these inter-trappean beds near Rajahmundry. While they are generally recognised as estuarine in character, W.T. Blanford[11] says : "The inter-trappean beds of Rajahmundry were deposited in brackish

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[10] *Quar. Jour. Geo. Soc. London*, 1921, 77, Part 3, 176.

[11] *Loc. cit. supra*, page 319.

water which was supplied with fresh-water by streams, but which was also in communication with the sea." The idea that the sea has played an important part in the deposition of some of these inter-trappean beds is supported by the fact that we have recently discovered the remains of numerous Foraminifers in these beds near Pungadi and Dudukur. A detailed study of these is in progress but enough work has been done to indicate the general character of this Foraminiferal fauna. The following families are commonly represented; Miliolidæ, Lagenidæ, Nonionidæ, Rotalidæ and Anomalinidæ; and in some cases these forms are so crowded that the rock may almost be called a Foraminiferal limestone. Since many of these families of Foraminifers have a fairly wide range in time, they are not of much value in determining the exact age of the beds containing them; but there is no doubt that a further study of these and other fossils in these marine intercalations in the Rajahmundry area will be of great value in comparing them with the sediments of the Trichinopoly area in the south and the Assam area in the north since all these areas must have formed part of the same marine province which came into existence as a result of the transgression of the southern sea, as will be obvious from a reference to the map showing the geography of Gondwanaland during the cretaceous period drawn by Dr. Fox and found accompanying his memoir[12] on "The Gondwana system and related formations".

The relation of the Rajahmundry area to the main mass of the Deccan traps.

We shall now proceed to briefly compare the position in Rajahmundry with what obtains in the main area of the Deccan traps in Central Provinces. In this latter area the lowest trap flows are frequently seen to overlie unconformably the Lameta beds or sometimes the Bagh beds; as a matter of fact, in a large part of this area the infra-trappean beds are the Lametas, which are believed to be the fresh-water equivalents of the marine Bagh beds (or slightly younger) and are of Cenomanian age. The Lametas have been so commonly noticed as the infra-trappean beds throughout this area that one working here develops a tendency to consider the two terms—Lametas and infra-traps—as synonymous and interchangeable. This is rather misleading at times, especially when one leaves the Central Provinces and goes to an altogether different area of the traps like that of Rajahmundry; for in this area immediately he sees the beds below the traps—the infra-traps—he starts with the assumption that these must be equivalent in all respects to the Lametas he has noticed as the infra-traps in Central Provinces[13] while,

[12] *Mem. Geo. Sur. Ind.*, 1931, **58**.

[13] For example, in his paper 'On the age of the fossiliferous beds at Dudukur'. (*Quar. Jour. Geo. Min. Met. Soc. Ind.*, Vol. 5, No. 2, June 1933, the late Prof. H. C. Das-Gupta says:

as a matter of fact, these beds of the Rajahmundry area may have nothing to do (and actually these have nothing to do) with the true Lametas, except that they are both infra-trappean in position.

In this connection we may point out that W. T. Blanford has carefully guarded himself against this tendency for he says[14] after describing the infra-trappean bed near Dudukur : "It is difficult to say whether this bed should be referred to the Lameta group or not. The mineral character is similar, but all known Lameta outcrops are so distant that the identification is somewhat doubtful. The distinctions between the fossils of the Bagh beds and those of the infra-trappeans of Dudukur and Pungadi appear to be too great to be attributed solely to the existence of a land barrier between the two areas ; it is difficult to suppose that the two formations can be of the same geological age ; and the difficulty consequently arises that if the Lameta beds represent the Bagh group, they are probably more ancient than the Pungadi infra-trappeans." We think it is very necessary to keep this distinction in mind, especially when we are comparing the infra-trappean beds of widely separated areas.

Now to come back to the position in Central Provinces. As mentioned above, in many parts of this area the lowest trap flows overlie the Lameta beds and are therefore younger. The question is, what exactly is the age of these traps ? Here again we have luckily a few inter-trappean beds with important fossils ; and it is from these beds found in the Nagpur-Chhindwara region that Dr. Sahni and his collaborators have described a rich silicified flora[15] and have shown that there is a considerable body of direct palæo-botanical evidence distinctly in favour of a tertiary age for these inter-trappean beds ; and in view of the fact that all these plant fossils come from the lowest part of the inter-trappean series, Dr. Sahni[16] naturally goes on to conclude that " the tertiary era had already dawned when the first lavas of the Deccan were poured out" and that "the whole period represented by the traps from Nagpur as far as the west coast was comprised within the lower tertiary and probably within the eocene."

In this connection we may also refer to the occurrence of certain fossil fishes discovered long ago in the supposed Lameta beds near Dhamni and

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"King placed the Dudukur fossiliferous band as infra-trappean. * * The beds known as infra-trappean with respect to the Deccan trap are, in terms of Indian Geology, designated as belonging to the Lameta stage. * * The infra-trappean and the Lameta beds are synonymous," pages 65-66.

[14] *Loc. cit. supra*, page 317.

[15] *Proc. Ind. Sci. Congress*, Botany section, Bombay. Read January 1934, published March 1934.

[16] *Curr. Sci.*, March 1935, 3, No. 9, 430.

Dongargaon, in Central Provinces. These fishes have been studied by Sir Arthur Smith Woodward[17] who after discussing their affinities fixed the age of this fish fauna as between the Danian cretaceous and the upper eocene, and thought they were probably of lower eocene age, a conclusion difficult to reconcile with the otherwise accepted Cenomanian age of the Lameta beds. But from a recent study of these fish-bearing beds near Dongargaon, Dr. C.A. Matley[18] has adduced reasons to believe that these "beds are not Lametas but are of inter-trappean age,".....laid down in a separate basin of deposition. This important conclusion clarifies the whole situation; and the fish fauna of the Dongargaon beds may now be considered as further supporting the case for the lower eocene age of the inter-traps in this area based on the plant fossils recently described by Dr. Sahni.

It has been generally admitted that these lava flows of the Nagpur-Chhindwara area, as also those of Rajahmundry, belong to the base of the Deccan trap formation as a whole; and in both these areas the fossil evidences from the lowest inter-trappean beds indicate that "the tertiary era had already dawned when the first lavas of the Deccan were poured out."

Conclusion.

It must be remembered, however, that practically all this palæontological evidence in support of an eocene age for the Deccan trap comes from plant fossils; and the question naturally arises as to what dependence is to be placed upon fossil plants as a guide to geological age—how far they could be considered "absolute indicators of a geological horizon". Whenever there has been a possible difference of opinion on the age of a bed as determined by the evidence of terrestrial fossil plants as against fossil marine animals, it is true that the geologist will always prefer the evidence of the latter and fix the age accordingly. But in a case like the age of the inter-traps where the decisive evidence of fossil marine animals is not available, it seems only reasonable to accept an age based on the verdict of the plant fossils, unless such a conclusion can be shown to be definitely unacceptable from the geological side.

Acknowledgments.

We are greatly indebted to Prof. Julius Pia, Ph.D. (of the Natural History Museum, Vienna) for the identification of *Acicularia*; and to F. Chapman, F.R.M.S., for his valuable assistance in the identification of the Foraminifera. Our best thanks are also due to the Director, Geological Survey of India, for his kindness in helping us with valuable literature.

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[17] *Pal. Ind. N. S.*, 1908, **3**, 1-6.

[18] *Rec. Geo. Sur. Ind.*, 1921, **53**, Part 2, 159.

THE MYXOPHYCEAE OF THE UNITED PROVINCES, INDIA.—II.*

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(Communicated by Prof. Y. Bhâradwâja, M.Sc., Ph.D. (Lond.), F.L.S.)

This communication deals with some Myxophyceæ (hitherto unrecorded with the exception of *Stichosiphon indica* Rao) from Benares. Three forms collected from Chakia (Benares State) have also been included. In all twenty-five forms, representing thirteen genera, have been described, and out of these two are new species, five are new varieties and six are new forms.

Systematic Enumeration of the Species Observed.

I. Chroococcales.

Chroococcaceæ.

Genus *Aphanothece* Nägeli.

1. *Aphanothece saxicola* Näg. Frémy, Les Myxophycées de l'Afrique équatoriale française, *Arch. d. Bot.*, iii, (1929), Mem. 2, Fig. 28, 1930.

Forma.

Lat. cell., 2–2·8 μ; long. cell., 3–6·8 μ.

Habitat :—Planktonic in road-side drains.

This form differs from the type in the cells being broader.

2. *Aphanothece pallida* (Kütz.) Rabenh. Frémy, *op. cit.*, 1930, Fig. 31.

Plant-mass forming a wide thick expanse, soft, transparent, pale yellowish-brown.

Lat. cell., 5–6·9 μ, rarely up to 8 μ; long. cell., 7·7–12·2 μ.

Habitat :—On wet soil.

3. *Aphanothece Nægelii* Wartm. Frémy, *op. cit.*, 1930, Fig. 24.

Lat. cell., 3·5–4·5 μ; long. cell., 6·5–7·8 μ.

Habitat :—On moist bricks.

4. *Aphanothece microscopica* Näg. Geitler, in Rabenhorst's *Kryptogamenflora von Europa*, XIV Band, Cyanophyceæ, 1930–32, p. 173, Fig. 79; Tilden, Minnesota Algæ, I, 1910, Pl. II, Fig. 12.

Lat. cell., 4–5 μ; long. cell., 6–9 μ.

Habitat :—Planktonic in a stagnant pond.

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* The first paper of this series was published in the *Proceedings of the Indian Academy of Sciences*, 1935, 2, No. 1, Sec. B.

Genus *Chroococcus* Nägeli.

5. *Chroococcus minimus* (Keissl.) Lemm. Lemmermann, *Kryptogamenflora d. Mark Brandenburg*, III, Algen, 1910, p. 59; Geitler, *op. cit.*, 1930–32, p. 233.

var. *crassa*. var. nov. (Fig. 1, *A*).

Plant-mass membraneous, soft, pale blue-green. Cells spherical, subspherical or ellipsoidal. Single or in more or less rounded or ellipsoidal colonies of 2–8 (rarely upto 12) cells which usually overlap. Sheath distinct, hyaline and stratified.

Diam. colon. cum vag., 4·5–10 μ; long. colon. cum vag., 4–10 μ, rarely upto 14·2 μ; lat. cell., 2–4·5 μ; long. cell., 2–6·5 μ; lat. cell. cum vag., 4·5–8 μ; long. cell. cum vag., 4·5–9·5 μ.

Habitat:—On moist bricks.

The alga differs from the type in having comparatively larger cells and stratified sheath. It further contrasts in being a terrestrial form.

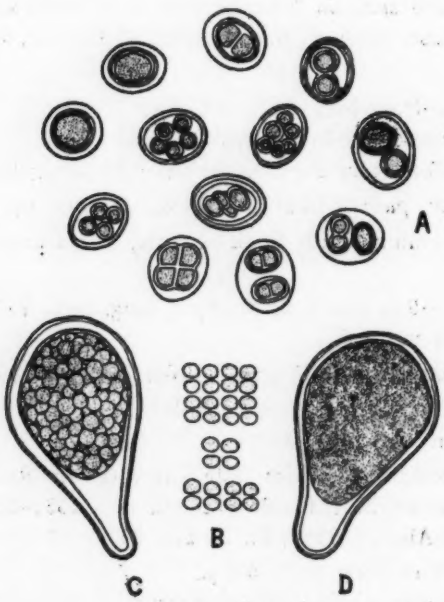

FIG. 1. *A—Chroococcus minimus* (Keissl.) Lemm., var. *crassa* var. nov.; *B—Merismopedia minima* G. Beck; *C* and *D—Dermocarpa olivacea* Tilden var. *gigantea* var. nov. *A* × 910; *B* × 3,175; *C* and *D* × 415.

Genus *Merismopedia* Meyen.

6. *Merismopedia minima* G. Beck. Geitler, *op. cit.*, 1930–32, p. 263, (Fig. 1, *B*).

Plants forming colonies usually of 4–8 (rarely upto 16) cells with pale blue-green or hyaline homogeneous contents.

Lat. cell., ·5–·6 μ.

Habitat :—Planktonic in a tank.

Genus *Rhabdoderma* Schmidle et Lauterborn.

7. *Rhabdoderma Gorskii* Wolosz. Geitler, *op. cit.*, 1930–32, p. 277, Fig. 135, *C*.

Lat. cell., 1·5 μ ; long. cell., 10–13 μ.

Habitat :—Planktonic in a rain-water pool.

II. Chæmosiphonales.

Dermocarpaceæ.

Genus *Dermocarpa* Crouan.

8. *Dermocarpa olivacea* (Reinsch) Tilden. Tilden, *op. cit.*, 1910, Pl. III, Figs. 26 and 27.

var. *gigantea* var. nov. (Fig. 1, *C* and *D*).

Plants single, broadly club-shaped, contracted at base to form an elongated stalk. Cell-wall thick, faintly stratified. Endospores numerous.

Lat. cell. cum vag., 45–56 μ ; long. cell. cum vag., 70–100 μ ; lat. cell., 20–43 μ ; long. cell., 33–76 μ.

Habitat :—On a species of *Cladophora* along with *Stichosiphon indica* Rao in a waste-water drain.

This alga resembles the type in the presence of broadly club-shaped cells surrounded by a thick stratified wall which is contracted at the base to form a stalk-like structure, and in the presence of numerous endospores ; but it differs in its much bigger size and in its being a fresh water form occurring singly.

Genus *Stichosiphon* Geitler.

9. *Stichosiphon indica* Rao. Rao, A new species of *Stichosiphon* (*Stichosiphon indica*), *Proceedings of the Indian Academy of Sciences*, Vol. II, 1935, p. 536, Figs. 1–10.

Lat. sporang., 6·4–8·6 μ ; long. sporang., 75–175 μ (average about 140 μ) or rarely up to 320 μ ; lat. spor., 5·5–7·5 μ ; long. spor., 6–14 μ ; diam. spor., 5·5–7·5 μ ; crass. vag., upto ·8 μ.

Habitat :—In a waste-water drain.

III. HORMOGONEALES.

(*a*) *Rivulariaceæ*.

Genus *Glœotrichia* Agardh.

10. *Glœotrichia intermedia* (Lemm.) Geitler. Geitler, *op. cit.*, 1930–32, p. 635.

var. *kanwaense* var. nov. (Fig. 2, *A–C*).

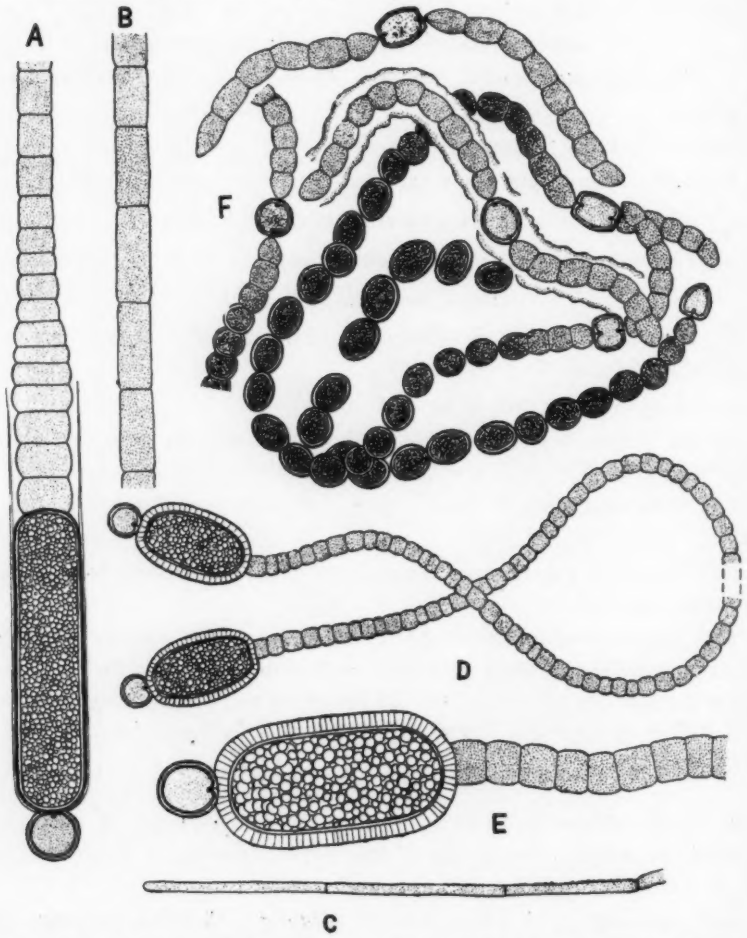

FIG. 2. *A*—lower, *B*—middle, and *C*—terminal portions of the filament of *Glœotrichia intermedia* (Lemm.) Geitler var. *kanwaense* var. nov.; *D*—entire plant and *E*—lower portion of the filament of *Cylindrospermum indica* sp. nov.; *F*.—*Nostoc spongiæforme* Ag. var. *tenuis* var. nov. *A-C* × 910; *D* × 910; *E* × 2,000; *F* × 910.

Plant-mass spherical, soft. Filaments with a thin hyaline and closely-adpressed sheath. Trichomes with constrictions at the joints. Cells at the base of the trichome invariably flattened and barrel-shaped, higher up almost quadratic or cylindrical, in the hair long cylindrical; with pale blue-green contents. Heterocysts, spherical or sub-spherical, usually single, rarely in pairs. Spores cylindrical, with smooth outer wall.

Diam. strat., 1–10 mm.; diam. trich., at base 10–13 μ, higher up 4–6 μ; long. cell., at base 2·5–4·5 μ, higher up 5–16 μ; lat. het., 10·2–13·5 μ; long. het., 8·6–11·5 μ; lat. spor. cum vag., 14–17 μ; lat. spor., 12–15 μ; long. spor., 55–66 μ; crass vag., ·8–1 μ.

Habitat :—In rain-water pools.

This variety agrees with the type in (1) the form and size of the plant-mass, (2) the presence of a thin, hyaline, and closely-adpressed sheath, (3) the occurrence of single (rarely two) almost spherical heterocysts, and (4) the form and size of the spores with smooth and colourless outer membrane; but it differs in the terminal hair of the trichome being straight, in the cells in the basal portion of the trichome being always broader than long, and in the spores being on the average comparatively shorter.

11. *Glœotrichia natans* Rabenh. Geitler, *op. cit.*, 1930–32, Fig. 406; Frémy, *op. cit.*, 1930, Fig. 246.

Lat. trich., at the base upto 12 μ, higher up 5–7·5 μ; long. cell., at the base 8–10 μ, higher up 5–20 μ; lat. het., 8–12 μ; lat. spor. cum vag., upto 30 μ; lat. spor., 10–18 μ; long. spor., 40–95 μ.

Habitat.—Attached to aquatic plants in a stagnant pond.

(*b*) *Nostocaceæ.*

Genus *Cylindrospermum* Kütz.

12. *Cylindrospermum indica* sp. nov. (Fig. 2, *D* and *E*).

Trichomes single, with deep constrictions at the joints, dark blue-green. Cells almost quadratic or more or less barrel-shaped. Heterocysts spherical, sub-spherical, sub-conical or ellipsoidal; one at each end of the trichome. Spores almost ellipsoidal, sometimes cylindrical; subterminal at either end of the trichome, with a thick and yellowish-brown outer membrane possessing a smooth outer margin and exhibiting faint lines running across in optical section.

Lat. trich., 3·7 μ; long. cell., 3–4·5 μ; lat. het., 2·8–5·8 μ; long. het., 3–7·6 μ; lat. spor. sine membr. ext., 8·8–9 μ; cum membr. ext., 10–12 μ; long. spor. sine membr. ext., 15–18·5 μ; cum membr. ext., 18–22 μ; crass membr., 1·5 μ.

Habitat :—In earthen water-pans in a green house.

This species approaches *Cylindrospermum trichotospermum* Frémy, in the breadth of the trichomes, the length of the cells, and in the dimensions of the ellipsoidal spores ; but it differs in the presence of smaller heterocysts and uniformly thick outer membrane of the spores. The alga may also be compared with *C. punctatum* Woronich. in the breadth of the trichomes and in the dimensions of the spores, but it differs in having shorter cells, bigger heterocysts, more or less ellipsoidal, single and subterminal spores with a smooth outer membrane. Again, the breadth of the trichomes and the presence of almost ellipsoidal heterocysts and spores (the latter exhibiting radial lines, though faint, in their outer membrane) in the present alga recall *C. alatosporum* Fritsch. But, the Benares form differs from the South African one in having shorter cells, much shorter and spherical or sub-spherical heterocysts without any shedding of the membrane, and in the single, subterminal and much shorter spores at either end of the trichome, enclosed all round by a thinner outer membrane, which is not shed.

Genus *Nostoc* Vaucher.

13. *Nostoc spongiæforme* Ag. Frémy, *op. cit.*, 1930, Fig. 279 *a* and *b* ; Tilden, *op. cit.*, 1910, Pl. VII, Figs. 4 and 5.

var. *tenuis* var. nov. (Fig. 2, *F*).

Plant-mass small, gelatinous, thin, expanded, brownish-black to brown ; sheath hyaline or pale yellow, usually more or less diffluent, occasionally firm. Trichomes densely entangled. Cells spherical, sub-spherical or barrel-shaped, rarely cylindrical ; those adjoining heterocysts slightly drawn out ; end-cell usually with pointed apex ; Heterocysts spherical, sub-spherical, ellipsoidal or barrel-shaped, rarely cylindrical with flat or rounded ends. Spores in chains of 3–15, spherical, sub-spherical or ellipsoidal, with a smooth hyaline outer membrane.

Lat. cell., 3–3·8 μ ; long. cell., 2·8–6 μ ; lat. het., 4–6 μ ; long. het., 5·5–9 μ ; lat. spor., 4·8–7·5 μ ; long. spor., 4·5–9 μ.

Habitat :—On moist walls.

This variety agrees with the type in the presence of (1) the barrel-shaped and cylindrical cells, (2) the spherical, barrel-shaped and ellipsoidal heterocysts, and (3) the ellipsoidal spores in chains with a smooth membrane. It, however, differs in having narrower trichomes and heterocysts, pointed apices of the end-cells, and much smaller spores (that are also spherical and sub-spherical) with a hyaline outer membrane.

Genus *Anabæna* Bory.

14. *Anabæna variabilis* Kütz. Frémy, *op. cit.*, 1930, Fig. 294.

Forma.

Lat. trich., 2·5–6 μ ; long. cell., 2·5–6 μ ; lat. het., 4·5–7·1 μ ; long. het., 6·3–9·6 μ ; lat. spor., 6–8·5 μ ; long. spor., 6·5–11·5 μ.

Habitat :—On moist soil, along with other algæ.

The form differs from the type in the presence of thinner trichomes, occasionally cylindrical spores, and heterocysts of variable dimensions.

(*c*) *Oscillatoriaceæ.*

Genus *Oscillatoria* Vauch.

15. *Oscillatoria subbrevis* Schmidle forma *major* G. S. West. Report on the Fresh-water algæ, including Phytoplankton, of the Third Tanganyika Expedition conducted by Dr. W. A. Cunnington, 1904–1905, *Journal of the Linnean Society, Bot.*, 1907, **38**, p. 178.

Lat. trich., 9·5–10 μ ; long. cell., 1–1·8 μ.

Habitat :—On moist soil.

The presence of bent septa, some convex and some concave to the apex of the trichome, as recorded by West, is only rare in the Benares form.

16. *Oscillatoria princeps* Vauch. Geitler, *op. cit.*, 1930–32, p. 946, Fig. 601 *c-g*.

Lat. trich., 42·9–43·5 μ ; long. cell., 4–4·5 μ.

Habitat :—In stagnant water of road-side drains in the Cantonment.

Another form collected from the University area possesses trichomes 21–23·5 μ broad.

17. *Oscillatoria terebriformis* Ag. Geitler, *op. cit.*, 1930–32, Fig. 607 *d*.

Lat. trich., 4–6 μ, at the apex 3 μ ; long. cell., 2·5–5·5 μ.

Habitat :—On the rocky dam near the source of a canal (Latif Shah, Benares State).

Forma.

Lat. trich., 4–6·5 μ ; long. cell., 2·5–5 μ.

Habitat :—On stones submerged in shallow water.

This form agrees with the type in all respects except that the ends of trichomes are simply bent but not twisted, and the old trichomes possess slight constrictions at the joints.

18. *Oscillatoria variabilis* sp. nov. (Fig. 3, *A–D*).

Plant-mass thin, membraneous, firm, pale blue-green to pale bluish-violet. Trichomes straight, usually of uniform thickness, and only rarely

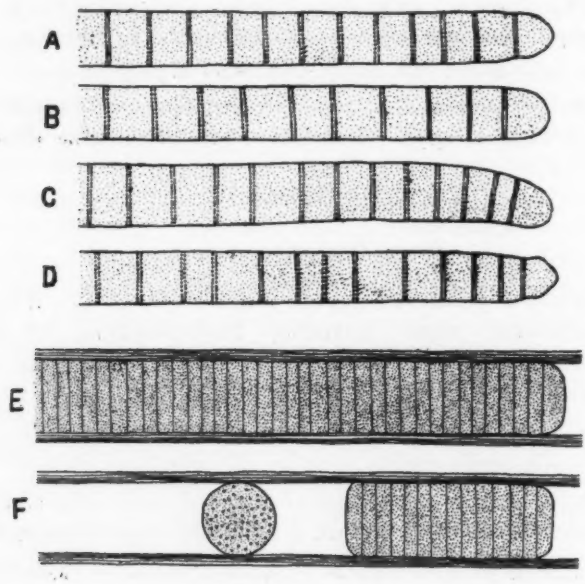

FIG. 3.

FIG. 3. *A—D, Oscillatoria variabilis* sp. nov.; *E—F, Lyngbya majuscula* Harv. var. *chakiaense* var. nov. *A-D* × 1,585; *E-F* × 335.

slightly tapering at the ends; without constrictions at the joints; septa indistinct but with distinct granules closely arranged on their either side. Cells with homogeneous contents; end-cell rounded, rarely conical, sometimes with constriction at the septum, without any calyptra.

Lat. trich., 5·2–6 μ; long. cell., 2·5–6 μ (average 5 μ).

Habitat :—Planktonic, along with other algæ in a rain-water pool.

This plant approaches *Oscillatoria Martini* Frémy in the breadth of the trichomes and in the absence of constrictions at the joints. It also resembles *O. Boryana* Bory in the breadth of the trichomes, in the length of the cells and in the presence of granules by the side of the septa. But it differs from both the above species in possessing straight trichomes of more or less uniform thickness with indistinct septa. It further differs from *O. Martini* Frémy in possessing longer cells, in the presence of granules on either side

of the indistinct septa and in the absence of both the cap and the thickening at the end-cells.

The present alga can also be compared with *O. granulata* Gard. and *O. Grunowiana* Gom. with which it resembles in the presence of rounded end-cells and granules on either side of the septa; but it differs from both in the trichomes being straight and broader, and in the presence of indistinct septa. It is further distinct from *O. Grunowiana* Gom. in the absence of any cap at the apex of the end-cell. The Benares alga, while further agreeing with *O. granulata* Gardner in the length of the cells, contrasts with the same in the absence of a thickening at the rounded end-cell. The Benares alga may also be compared with *O. Koellitzii* Fritsch on account of the presence of indistinct septa with small granules on their either side, but it differs from the same in the possession of narrower trichomes and longer cells and in the absence of a rather conspicuous cap at the apex of the end-cell.

19. *Oscillatoria Agardhii* Gom. Geitler, *op. cit.*, 1930–32, Fig. 621 *a–h*.

Lat. trich., 4–6 μ; long. cell., 2·5–4 μ.

Habitat :—Planktonic in a pool.

This form differs from the type in the absence of gas-vacuoles.

Genus *Phormidium* Kütz.

20. *Phormidium Jadinianum* Gom. Frémy, *op. cit.*, 1930, p. 137, Fig. 118.

Forma.

Lat. trich., 4–6 μ; long. cell., 1·3–2·8 μ.

Habitat :—On moist soil.

This form differs from the type in the trichomes possessing shorter cells and terminating with bluntly-rounded end-cells.

21. *Phormidium Bohneri* Schmidle. "Beitrage zur Algenflora Afrikas," *Engler's Botanische Jahrbucher*, 30, 1902, Taf. II, Fig. 11.

Forma.

Lat. fil., 1·7–2 μ; long. cell., 1·5–2·1 μ.

Habitat :—On a damp wall.

This form differs from the type in the trichomes being irregularly curved.

22. *Phormidium ambiguum* Gom. Geitler, *op. cit.*, 1930, p. 1012, Fig. 647 *e*.

Lat. trich., 4–6 μ; long. cell., 1·3–2·5 μ.

Habitat :—On a moist wall.

Genus *Lyngbya* C. Agardh.

23. *Lyngbya ceylanica* Wille. Frémy, *op. cit.*, 1930, p. 185, Fig. 153.

Forma.

Lat. fil., 10·8–11·5 μ; lat. trich., 9·8–10·5 μ; long. cell., 6–8·5 μ; lat. vag., 0·5–0·8 μ.

Habitat :—Free-floating in shallow water near a dam (Latif Shah, Benares State).

This form differs from the type in having longer cells and thinner hyaline sheath.

24. *Lyngbya majuscula* Harv. Frémy, "Les Cyanophycées des Côtes d'Europe," *Memoires de la Société Nationale des Sciences Naturelles et Mathématiques de Cherbourg*, tome XLI, 1934, pl. 28, Fig. I *b*; Geitler, *op. cit.*, 1930–32, p. 1060, Fig. 672 *c* and *d*.

var. *chakiaense* var. nov. (Fig. 3, *E* and *F*).

Plant-mass loose, bluish-black to pale yellowish-brown; Filaments deep blue-green to almost violet, straight or irregularly curved and entangled. Sheath thin, hyaline, stratified, with more or less uniform outer margin, unstained with Chlor-zinc-iodide. Cells flattened or disc-shaped, with finely granular contents; end-cell flatly rounded. Harmogones consisting of one to many cells; single-celled harmogones often getting turned over and presenting their flat surfaces in optical section.

Long. fil., upto 3 cm.; lat. fil., 33·5–50 μ; lat. trich., 25–39·5 μ; long. cell., 4–7·6 μ (average about 6 μ); crass. vag., 3·8–5·2 μ.

Habitat :—Free-floating in shallow water near a dam (Latif Shah, Benares State).

This variety agrees with the type in the presence of the long irregularly curved and deep blue-green filaments, in the hyaline stratified sheath, and in the broadly rounded end-cell; but it differs in the cells being longer and the sheath being much thinner.

Geitler (*op. cit.*, 1930-32, p. 1067) mentions an affinity between *Lyngbya majuscula* Harv. and *L. magnifica* Gard., but the alga in question differs from the latter in having broader filaments, longer cells, and thicker stratified sheath. It further differs in the absence of a thickening at the apex of the end-cell.

In conclusion, I have great pleasure in expressing my great indebtedness to Professor V. Bhâradwâja, for his kind guidance and criticism throughout the course of this investigation.

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ON THE POSSIBLE EFFECT OF THE ENVIRONMENT ON THE CYTOPLASMIC INCLUSIONS IN THE OOCYTES AND OOGONIA OF *DASYCHONE CINGULATA, SALMACIS BICOLOR*, AND *CLIBANARIUS OLIVACEUS*.

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Received February 3, 1936.

Introduction.

Studies on the cytoplasmic phenomena during oogenesis in the same animal by different authors (for example *Lumbricus* Harvey, 1925; Gatenby and Nath, 1926; *Lithobius* King, 1924; Nath, 1924; Weiner, 1925; and Koch, 1925) have brought to light a remarkable diversity of results. Even a reinvestigation of these phenomena in the same animal by the same author (*e.g.*, *Lumbricus* Harvey, 1925 and 1931; *Pheretima* Nath, 1930 and 1933), shows no correlation. Taking *Lumbricus* Harvey in 1925 showed that there is a fair amount of yolk in the egg and he gives tests for distinguishing yolk from fat (pp. 299 and 306, 1925). But in his restatement of the oogenesis of *Lumbricus* he observes that the material, which he designated as yolk for want of a better term is really a weak fat. These fat droplets blacken in osmic, but in osmicated ovaries brought up through alcohols and mounted in balsam leave empty vacuoles. These according to him are able to resist alcohol only up to 70% dissolving in 90%. Nile blue sulphate stains these spheres very pale blue; in Sudan III they are pale yellow and after Scharlach R deep scarlet. Since these spheres were found to dissolve in 90%, how he was able to see them in finished slides (1925) and decide that they are yolk, giving at the same time tests, baffles one.

In *Pheretima* according to Nath (1930) the Golgi vesicles contain fat, and as a result in Champy fixed ovaries mounted whole appear as black granules, but within a month or so they are decolourised. In Champy fixed sections these vesicles are decolourised on immersion in Xylol.

Nath (1933) in his recent contribution (basing his observations on staining with Sudan III and Scharlach R) mentions that nothing in the oogonia or oocytes of *Pheretima* was stained by Sudan III or Scharlach R. He therefore, concludes that what the Golgi are composed of and also contain is a highly unsaturated or little oxidized lipoid.

Harvey (1925 and 1931) and Nath (1930 and 1933) in an attempt to correlate their results with the observations of other workers (Gatenby and Nath, 1926; Weiner, 1925 and 1930; Foot and Strobell, 1900 and 1901; Foot, 1894 and 1896) suggest that these differences may be due to the idiosyncrasies of the technique. For example, Harvey (1931) comes to the conclusion that what Nath (1930) considers as Golgi bodies in *Pheretima* are not Golgi bodies at all but that they are droplets already of a fatty nature. The view expressed by Harvey would appear to be untenable as Nath definitely states that the Golgi bodies in *Pheretima* appear as solid blackened granules after Da Fano (p. 495, 1930) and that even in Bouin preparations these distorted Golgi elements occur (p. 497) as spherules resembling yolk droplets. Harvey (1925) himself shows in the table extracted from the *Vade-Mecum* that fat vacuoles are not fixed in Cajal and that they are washed away by Bouin. Even Nath's interpretation of the fat content of Golgi vacuoles would appear to be erroneous because it is well known that Golgi containing fat cannot be seen as granules after Da Fano or preserved after Bouin. Further Nath (1933) doubts the existence of fat droplets, as reported by Harvey, as these give a negative reaction to Nile blue sulphate, a positive one to Scharlach R and a weak one to Sudan III. Lorrain Smith (1907) found that Nile blue sulphate stains neutral fats red and fatty acids blue. Tennent, Gardiner and Smith (1931) demonstrate that while triolein taken separately is stained red and oleic acid blue by Nile blue sulphate, mixtures containing a very small amount of oleic acid are coloured blue by this stain. The fat in eggs is not found in the pure state and as its composition may vary it is difficult to accept Nath's criticism. That Scharlach R and Sudan III cannot be depended upon is a very well-known fact and hence Nath's observations cannot be regarded as conclusive. And further, it would be interesting to know the action of turpentine on bodies that stain with Sudan III and Scharlach R.

The authors of the present paper feel from the results obtained that the main cause for differences in observation depends more upon other factors than on technique.

It has been shown by one of us in a previous paper (Subramaniam, 1935*a*) that differences in the physico-chemical factors of the environment is followed by differences in the quantity and mode of behaviour of the various inclusions. In the present paper we hope to dilate on those observations.

Literature.

Since Orton's work (1922) on "Sea Temperature, Breeding and Distribution in Marine Animals" innumerable papers have appeared showing the effect of temperature on breeding. In *Calanus finmarchicus* Russel

(1928) has shown that the generations vary not only in size but also in physiology. Bogorov (1934) has further demonstrated that there is an alteration in biomass and size during the various seasons. Biomass and size are small in February to mid-April and both reach a maximum in May. From June to December biomass and size remain stable after a slight decrease and the values reach their lowest level in October. Bogorov mentions that there is a definite relation between size and physico-chemical conditions of the environment, especially temperature. The results of Bogorov and Bogorov and Preobrajensky (1934) indicate that the average biomass of *Calanus* in Barent's Sea (where it produces only one generation a year) is equal to the weight of three generations of *Calanus* produced in the English Channel. The difference in temperature between 'spring' and 'autumn-winter' generation in the English Channel is about 8°C. and the biomass of 'spring' *Calanus* is twice as large as the 'autumn-winter' ones. The differences in temperature between English Channel and Barent's Sea is about 10°C. and the average biomass of *Calanus* in Barent's Sea is twice the value of that in English Channel. The northern and southern parts of the Barent's Sea show a difference of 5°C. and this difference brings about a three-fold difference in biomass. Life of *Calanus* in Barent's Sea is longer than that in the English Channel.

The breeding periods of *Echinus esculentus* (Moore, 1934) vary even in localities separated only by a few miles and at different depths. According to Moore (1934) temperature is the important factor that causes such variations.

Finally the chemical analyses of Plankton by Brandt (1898) and Raben and Brandt (1919) indicate a variation in the quantities of fat, protein and water content during different seasons. Orr (1934) finds that in *Calanus finmarchicus* from Loch Fyne, the fat content generally follows the weight curve but that it is highest at the beginning of March and lowest in May. Wimpenny (1929) working on the North Sea zoo-plankton records that maximum fat content was found in August and minimum in October. Similarly the percentage of protein in the body varies also during the different seasons (Orr 1934, Marshall, Nicholls and Orr, 1934). Orr further states that in March when fat content was unusually high a low value was obtained for protein content. The protein values varied from 50 to 35% and the percentage also followed the weight curve in general (Marshall, Nicholls and Orr, 1934).

A seasonal variation in the amount of glycogen and total carbohydrate content was noticed by Stott (1931) in *Echinus esculentus*, and he supposes that glycogen is transformed in maturing gonads into carbohydrate food

reserved for the ripe sperms and eggs. The records of Stott also show an individual variation in the fatty acid content of the gonads.

Bogorov (1934), Bogorov and Preobrajensky (1934), Moore (1934), Marshall, Nicholls and Orr (1934), Orr (1934), and Stott (1931) pay attention only to temperature. Temperature is but one of the factors. Pantin (1931 *a* and *b*), Weil and Pantin (1931), Beadle (1931), Schleiper (1929 *a* and *b*), Adolph (1925), Goldfarb (1914) and many others have found that salinity also affects the weight of an animal. Schleiper (1929 *a* and *b*) and Beadle (1931) show that such alterations are followed by changes in the respiratory rate. The effect of the environment is collective and all factors such as Temperature, Excess Base, Salinity, pH, Nitrate, Silicate and Ammonia content may influence in some way or other the metabolism of an animal.

The authors of the present paper have studied the environmental conditions and have attempted to correlate these with changes in the size, quantity, and variation of the inclusions observed by them in a few animals. Whether these changes are caused by any one factor or whether they are due to all of them is difficult to prove.

Methods and Materials.

Clibanarius olivaceus was obtained from the brackish waters at the mouth of the river Adyar and *Salmacis* and *Dasychone* from the Madras Harbour. Water samples on the days of collection were analysed and the following methods have been employed.

Hydrographical Technique.—

(1) All water analysis records given are of samples collected at 8 a.m. from the mouth of the river, as well as from the Harbour, irrespective of the nature of the tide.

(2) The temperature was determined by the aid of a surface temperature thermometer giving readings correct to 0.1°C. Each reading is the mean of three readings taken from places at approximately 50 yards from each other.

(3) The pH was determined with the aid of a Hellige Comparator.

(4) For the determination of salinity, both Knudsen's Chlorine Titration apparatus and Richter and Weiss' set of aerometers were used.

(5) The Excess Base was determined according to the method outlined by Bruce (1924) and the results obtained were checked from time to time by Saunders's method (1926).

Cytological Technique.—The material was fixed immediately it was brought into the Laboratory. The ovaries were removed—out of contact

Records of Sea and River Water Analysis.
December 1932—*July* 1933.

A	B Temperature		C pH		D Excess Base		E Salinity		F Chlorine		G Air Temperature	Difference between Air and Sea Temperature	Difference between Air and River Temperature
Month	Sea	River	Sea	River	Sea	River	Sea	River	Sea	River			
December ..	75·9°F.		8·41		18·7		28·73		15·90				
January ..	76·01	77·0	8·47	8·48	19·98	20·73	30·59	28·17	16·93	15·59	75·2°F.	plus 0·81	plus 1·8
February ..	79·16	79·16	8·5	8·55	20·25	23·2	31·94	30·44	17·68	16·85	75·6	,, 3·56	,, 3·56
March ..	82·94	81·86	8·49	8·41	19·55	17·59	33·88	29·92	18·75	15·56	78·3	,, 4·64	,, 3·56
April ..	83·12	82·76	8·5	8·46	20·2	25·16	34·48	17·29	19·09	9·56	84·5	minus 1·38	minus 1·74
May ..	85·28	85·37	8·65	8·65	19·9	24·9	34·81	19·74	19·27	10·92	86·8	,, 1·52	,, 1·43
June ..	83·48	83·84	8·48	9·07	19·78	24·52	35·39	16·91	19·57	9·35	84·5	,, 1·02	,, 0·66
July ..	83·48		8·43		20·72		35·16		19·46				
Total No. of Readings ..	16	15	29	24	25	22	18	15	18	15			

with any liquid—cut into small pieces and immediately transferred to the fixatives. Carnoy, Flemming, Flemming without acetic, Champy Kull, Regaud, Bensley-Cowdry, Nassonov's modification of Kolatschev's method, Mann Kopsch, Mann Kopsch Altmann, Ludford's modification of Mann Kopsch, Da Fano, Corrosive acetic and absolute alcohol were employed. The stains generally used were Heidenhain's Iron-Alum Hæmatoxylin, Orange G, Alum Carmine, Acid Fuchsin, Toluidin blue, Aurantia, Thionin, Methyl Green, Methyl Blue Eosin, Pyronin Methyl Green, etc.

In addition fresh eggs were studied before and after treatment with osmic acid. Ciaccio's test for lipoids as well as Scharlach R and Sudan III stains were also used.

From regular records of analyses of the Sea and River water, are given below the results for the months December to July. (See Table I.)

Conditions in the Brackish Water.

The conditions observed at the mouth of the river differ entirely in the two seasons. In January-February after the heavy rains of the South-West Monsoon, the bar is open, and the sea water mixes with the river water during high tide. As can be gleaned from the Table, the temperature of the water is near the minimum during these periods and the salinity is very high. The pH is also low in comparison with that for the April-June period and the same may be said of Excess Base. The readings for March—except in the case of temperature—are lower owing to the unusual rain of 3·42″ recorded for that month in Madras.

Conditions in the Period Mid-April to June.

From the middle of March the level of water in the river begins to fall with the consequent widening of the bar. The open river mouth becomes narrower and narrower and also silts up, and in the first week of April the sea invades the river only at high tide. Even this ceases in the middle of April, resulting in an entire change in salinity.

With the closing of the river, the salinity of the river water shows a sudden fall from 29·92 to 17·29. Changes also take place in the Excess Base and pH.

As can be seen from the Table the air temperature is near the maximum for the year in the months of April, May and June.

Conditions in the Sea.

The conditions observed in the Madras Harbour are, however, different. The temperature shows a steady rise from January to July. The pH values

fluctuate slightly, the minimum observed being in December and the maximum in May. In general it may be said that there is a steady increase from December to May after which there is a fall. The Excess Base shows no orderly rise probably due to the peculiar conditions existing in the Harbour. The salinity is very low in December as a result of the mixing of fresh water brought in by the river but shows a steady rise till June when it reaches the maximum. It will be noticed that in comparison with the records for the brackish water the 3·42″ of rain affects only the pH and Excess Base readings.

The temperature of the sea also follows the same course as that of the river being above that of the air in January, February and March and below in April, May and June.

Sizes of Eggs during the Two Seasons.

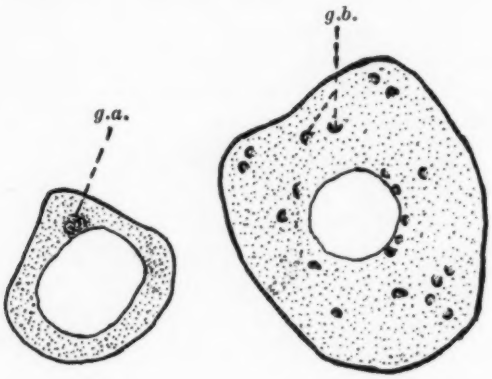
	Clibanarius		*Dasychone*		*Salmacis*	
	Jan.-Feb.	Apr.-Jun.	Dec.-Jan.	May.-Jun.	Dec.-Jan.	Apr.-Jun.
Average Size	302·8 μ	391·6 μ	85·6 μ	77·5 μ	102·91 μ	100·87 μ
Smallest fully developed egg..	246·6 μ	277·2 μ	69·3 μ	62·7 μ	79·2 μ	72·6 μ
Biggest egg	518·8 μ	569·8 μ	102·3 μ	99·0 μ	138·6 μ	122·1 μ

Bi-weekly measurements of 250 eggs on an average were continued throughout the period of investigation. It will be seen from the Table that whereas in the case of the marine animals there is a consistent decrease in size, in *Clibanarius* which is a brackish water form there is an increase. All these animals are exposed to the same high temperatures and hence the temperature factor may be eliminated. The notable changes are only in salinity and while in the sea the maximum is reached in May-June, in the river there is a sudden fall. It is presumed from the above results that the variation in the sizes of the eggs is caused by variations in salinity.

Oogenesis of Dasychone.

The Golgi Apparatus.—In the youngest oocytes studied the Golgi apparatus occurs as a few batonettes on one side of the nucleus (Fig. 1). In Fig. 2 is shown the spreading of the Golgi batonettes from this initial concentration. In December-January preparations individual batonettes migrate to various parts of the cytoplasm but in those in May-June (Fig.3) clumps of two or three first break off and the individuals constituting these later separate and become scattered throughout the cytoplasm. From the

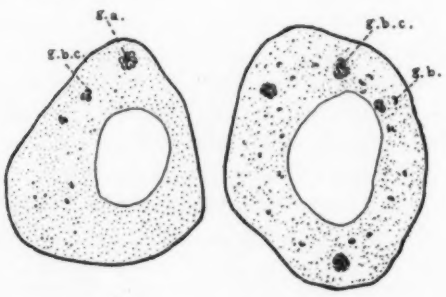
commencement of migration, these batonettes increase in number as could be made out from Figs. 1, 2 and 3. It has not been possible to ascertain whether the multiplication is by transverse or longitudinal fission but the orientation of these bodies suggests that it is longitudinal. Both in December-January and May-June oocytes multiplication appears to be rapid from the time the batonettes get scattered throughout the cytoplasm. In December-January especially the batonettes occur in clumps as shown in Fig. 4.



1. 2.

FIG. 1. Earliest stage of Golgi bodies. December-January. Da Fano. × 2,000.

FIG. 2. Spreading of the dictyosomes throughout the cytoplasm. December-January. Da Fano. × 2,000.



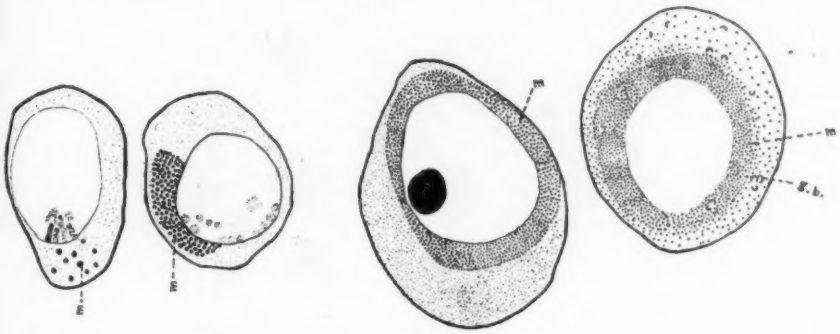
3. 4.

FIG. 3. Spreading of the dictyosomes in May-June. Da Fano. × 1,500.

FIG. 4. Multiplication of the dictyosomes. December-January. Da Fano. × 1,000.

The Mitochondria.—In Fig. 5 which is that of a very young oocyte, is shown the mitochondria, occurring as a few large grains on one side of the nucleus. These divide, become smaller and begin to spread around the

nucleus. In Figs. 6 and 7 are shown the formation of a circum-nuclear concentration by the mitochondria. The depth of this concentration increases probably due to division and the grains begin to migrate to the other portions of the cytoplasm (Fig. 8). The spreading is uniform and takes place all



5. 6. 7. 8.

Fig. 5. Earliest stage of mitochondria. *F. w. a.* Iron Hæmatoxylin. December-January. × 1,500.

Fig. 6. Multiplication stage of mitochondria. *F.w.a.* Iron Hæmatoxylin. December-January. × 1,500.

Fig. 7. Formation of a circum-nuclear concentration by the mitochondria. *F.w.a.* Iron Hæmatoxylin. December-January. × 1.500.

Fig. 8. Spreading of the mitochondria from the circum-nuclear concentration. Flem. *F. w. a.* Iron Hæmatoxylin. December-January. × 1,000.

over. In the fully grown oogonium (Fig. 9) the mitochondria are scattered uniformly throughout the cytoplasm.

In May-June preparations the mitochondria behave similarly but from the time of commencement of migration from the circum-nuclear concentration a sudden increase in size has been noticed. Figs. 9 and 10 which are camera lucida drawings under the same magnification show the difference in size. The mitochondria in May-June are almost ten times as large as those in December-January.

The Nucleolus.—The nucleolus occurs as a spherical body in the young oocytes (Fig. 7). It is not well fixed at all in December-January Da Fano and Bouin material. In May-June the distortion is comparatively less. There appear to be individual variations also. The nucleolus is darkened by osmic fixatives in both seasons but the blackening in December-January material can be extracted in a few minutes with turpentine. In May-June, extraction is negligible even after 6 days. The nucleolus begins to bud as

shown in Fig. 9. The mode of budding also exhibits individual variations. Only one or two buds at a time may be formed or a large number may be

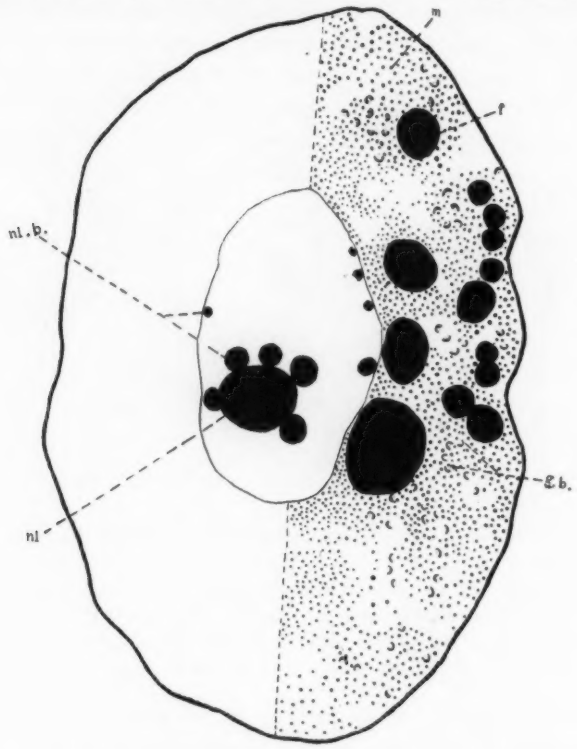
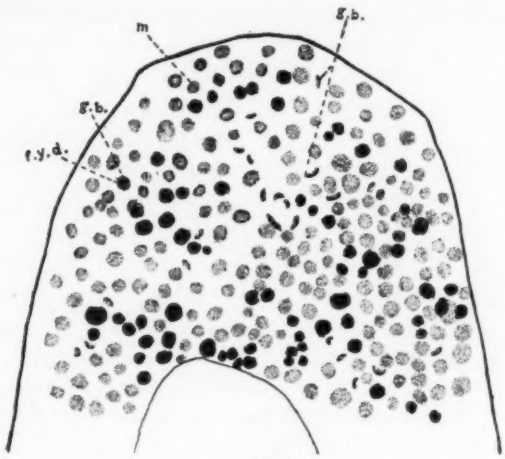


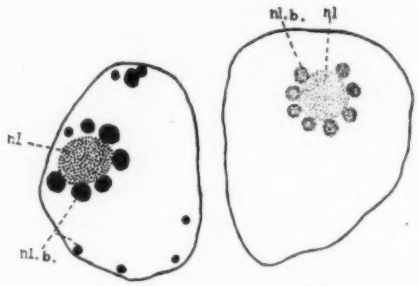
Fig. 9. An almost fully grown egg showing dictyosomes, fat vesicles, nucleoli and mitochondria. *F. w. a.* Iron Hæmatoxylin. December-January. × 1,000.

produced all round the nucleolus (Figs. 9, 11, 12). These buds also exhibit reactions similar to those of the original nucleolus but with this difference; in December-January the buds are not fixed at all in Bouin and Da Fano and the nucleolus is very much distorted, whereas in May-June the distortion is less and the buds are often preserved though much changed in appearance. Reactions of these buds and nucleoli to Sudan III and Scharlach R also differ in the two seasons. In December-January both are stained deep red (Fig. 11) while in May-June they (Fig. 12) stain orange of slightly varying shades. The nucleolar buds detach themselves from the main mass and pass to the nuclear membrane to which they firmly stick (Figs. 9 and 11). No rupture of the nuclear membrane has been observed but as the fat and fatty yolk is



10.

FIG. 10. A portion of an almost fully grown egg from a May-June preparation and showing the larger size of the mitochondria, two types of Golgi bodies, fatty yolk etc. Nassonov unstained. × 1,000.



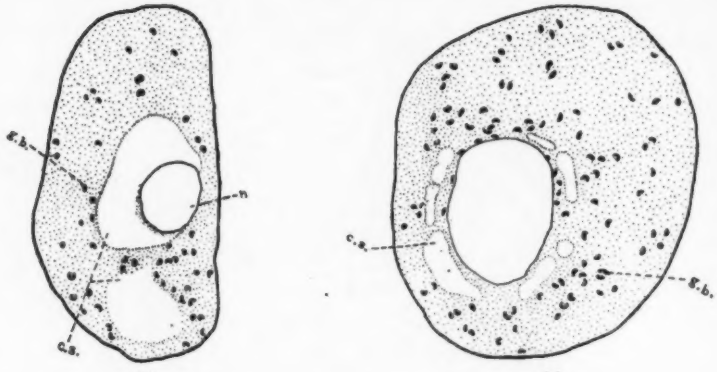
11. 12.

FIG. 11. Nucleus with the contained nucleoli. Both the nucleolus and the nucleolar buds are stained deep red by Sudan III and Scharlach R December-January. × 1,000.

FIG. 12. Indra vital staining of fresh eggs with Sudan III and Scharlach R in May-June. Nucleolus and nucleolar buds are stained in varying shades of orange. × 1,000.

formed near the nucleus the authors believe that the materials in the buds diffuse into the cytoplasm through the nuclear membrane.

Fat and Fatty Yolk.—It will be seen that in comparing Figs. 3, 4 and 13 there is a sudden decrease in the number of Golgi bodies (see Fig. 13) followed by the appearance of clear spaces. Both being Da Fano the possible explanation for such a phenomenon is that some of the Golgi have been converted into fat. These spaces also increase in number and size with the growth



FIGS. 13 and 14. Fat formation. Note the clear spaces left by the fat vacuoles. Da Fano. December-January. × 600.

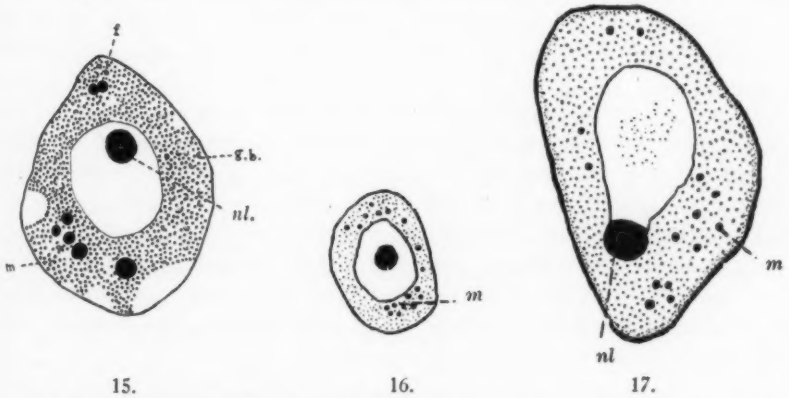
of the oocyte as can be seen from Figs. 13 and 14. Fig. 15 is from a Nassonov preparation and shows the first appearance of fat globules. These globules are black in osmic fixatives and are not preserved in Bouin, Corrosive acetic, Da Fano, etc. The sudden decrease in the number of Golgi suggests a transformation of the Golgi into fat and we are led to believe that each droplet of fat is formed by a group of Golgi bodies. The unmodified Golgi continue the division process started before and more and more Golgi bodies get transformed into fat. The diffusion of the nucleolar material begins after the division of the Golgi bodies comes to a standstill. The fat globules are near the nuclear membrane and the diffusion of nucleolar material is followed by an increase in the size of the fat globules. That some new vacuoles are also formed from the material of the nucleolar buds is suggested by the very great disparity in the size of the fat globules as also by the late origin of some of the smaller fat vacuoles. In the mature oogonium (Fig. 9) there are Golgi bodies, fat droplets and mitochondria in the December-January period. The fat droplets blackened after Champy and F.w.a. are dissolved out by the xylol in Canada balsam in the course of a fortnight after which they appear as clear vacuoles. In Nassonov and Mann Kopsch preparations such a quick dissolution is not apparent but even here the droplets look dull black after six months. Sudan III and Scharlach R stain these deep red and Ciaccio's test gives an orange colour for the Golgi and a red one for fat vacuoles.

Fatty Yolk.—In May and June, examination of fresh eggs, as well as fixed preparations, reveals the absence of any fat globules. There are only two inclusions in the young oocytes, the Golgi and the mitochondria. When nucleolar emissions begin, the Golgi which up till now were uniform in size

begin to get differentiated into two sets. Some grow larger in size than the others. In fresh eggs and fresh eggs osmicated for a short time the following reactions have been observed. In December-January oocytes and oogonia and in May-June earliest oocytes the chromophobe part of the batonettes becomes visible only after varying periods of osmication, being not visible in fresh eggs. In both cases the chromophobe part does not give any reaction to Ciaccio's test. But when in May-June growing oocytes, the Golgi begins to differentiate into two sets, namely larger and normal ones, in the former the chromophobe part becomes visible *intra vitam*. From the time of the beginning of differentiation Sudan III and Scharlach R stain this part orange. In osmic fixatives they are blackened (ref. Fig. 10 *f.y.d.*) but as in the case of the nucleolus and nucleolar buds in May the colour is not extracted by turpentine even after six days. In Champy and F.w.a. material xylol does not decolourise these portions. We consider these as the fatty yolk droplets, lipoidal in composition and infer that the material of these droplets is derived from the nucleoli which have diffused out and have been condensed by the Golgi. There is no transformation of Golgi.

Seasonal Variation in Salmacis.

In a previous paper one of us (Subramaniam, 1934) studied the oogenesis of *Salmacis bicolor*. The study was extended over a period of two seasons. The most striking facts observed were (1) a variation in the sizes of the oocytes when the mitochondria were first visible (Figs. 16 and 17)



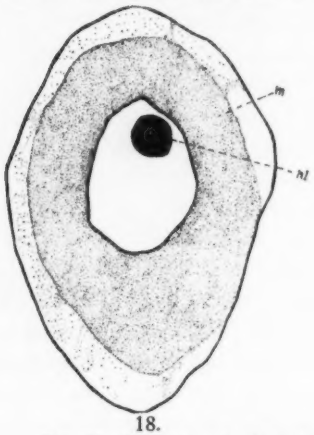
15. 16. 17.

Fig. 15. Beginning of fat formation. Nassonov. December-January. × 1,000.

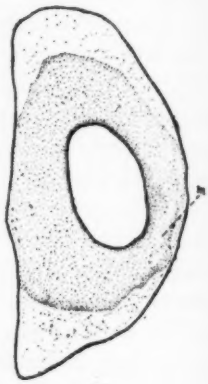
Fig. 16. *Salmacis*. Earliest stage of mitochondria. December-January. Flemming without acetic. Iron Hæmatoxylin. × 3,000.

Fig. 17. *Salmacis*. Earliest stage of mitochondria observed in preparations made in April. Flemming without acetic. Iron Hæmatoxylin. × 3,000.

and (2) a variation in the quantity of the mitochondria as also of yolk (Figs. 18 and 19). The nutritive bodies formed are fewer in April-May and a comparison of the figures 18 and 19 will show that the mitochondrial circum-nuclear concentration is very thin as against the very thick one in



18.



19.

Fig. 18. *Salmacis*. A stage just before the cytoplasm of the oocyte is filled up by the spreading mitochondria. An attempt has been made to represent the thickness of the mitochondrial concentration. *F. w. a.* Iron Hæmatoxylin. December-January. × 600.

Fig. 19. *Salmacis*. An oocyte at the same stage as in Fig. 18. Note the sparse distribution of the mitochondria. *F. w. a.* Iron Hæmatoxylin. × 600.

December-January. Yolk spherules formed are also very much less in May in correlation with the smaller quantity of the mitochondria and nutritive spheres.

Seasonal Variation in Clibanarius.

Oogenesis of *Clibanarius olivaceus* (Henderson) worked out by Subramaniam (1935 *a*) has revealed the existence of a difference in the mode of formation of albuminous yolk and in the amount of both fatty and albuminous yolk. In January-February oocytes there is a voluminous formation of fatty yolk and albuminous yolk of Golgi-mitochondrial origin alone has been observed. In April-June on the other hand fatty yolk formation is less and that of albuminous yolk more and two varieties of albuminous yolk—Golgi-mitochondrial and Golgi-Golgi—occur.

Discussion.

It will be seen from the material presented in this paper that the environmental factors as they change affect the size, quantity, behaviour and time of appearance of these inclusions. Work on *Meretrix casta** which has already

* *Journal of Morphology* (in press).

been completed in this Laboratory shows that variations in the behaviour and manner of spreading of the Golgi bodies are considerable. In addition, in the three forms described in this paper we get a pronounced seasonal variation which is all the more remarkable.

This leads to the general questions (1) why such variations occur during different seasons of the year and (2) whether similar variations may be expected in animals collected from different parts of the world.

We are led to believe that these variations in the behaviour and quantity of the inclusions in correlation with seasonal changes in the physico-chemical factors of the medium represent variations in metabolism. The observations recorded in the case of *Clibanarius*, *Dasychone* and *Salmacis* allow of no other interpretation. This is further supported by the results obtained by Bogorov (1934), Bogorov and Preobrajensky (1934), Moore (1934), Orr (1934), Marshall, Nicholls and Orr (1934), and Russell (1928). To the cytologist the cell is the unit and variations in the metabolism in the animals are only expressions of the changes in the cell groups under different conditions. Does not this interpretation explain the somewhat diverse results obtained by workers on the same animal collected from the same locality ?

Bogorov's and Bogorov and Preobrajensky's results throw some light on the cause of the varying results obtained by workers in different parts of the world. Further evidence in support of our view, *viz.*, that differences in the environment, whether seasonal or geographical, are probably due to changes in the metabolism of the animals, is provided by the different conclusions arrived at by different workers in the case of *Lithobius*.

Reverting now to the cell, recent work has shown clearly that the mitochondria and the Golgi bodies are concerned in the yolk or fat formation in the eggs of various animals. If the Golgi bodies are the active agents in the synthesis of fat or yolk and if such a conclusion is based on topographical as well as diagnostic characters, it is reasonable to expect a similar function in cells where there is no fat or yolk. The scattering of the parts of the Golgi apparatus during cell division has been taken to represent an increase in the metabolism of the cell, and hence the uniform distribution of the Golgi bodies from an initial concentration in the egg in which there is no yolk or fat, indicates an attempt by the apparatus to cope with the increasing metabolism of the cell. How metabolism in the cell is controlled has baffled all interpretation. Theoretical as well as experimental evidence (Subramaniam, 1934, 1935 *a*, 1935 *b*, 1935 *c* : oogenesis of *Meretrix casta* (in press) ; Subramaniam and Gopala Aiyar, 1935 *a* and 1935 *b*) has been offered in previous papers for concluding that the Golgi apparatus is the fountain-head of the various enzymes. And, on the basis of such an explanation, it becomes

increasingly evident why there should be variations in the behaviour of the apparatus with changes in metabolism.

Summary.

1. The oogenesis of *Dasychone* and *Salmacis* has been worked out from oocytes collected during two different seasons.

2. Changes in the physico-chemical factors of the Sea and the Adyar river water have been studied.

3. Measurements of the ripe oocytes indicate a consistent increase in size in the oocytes of *Clibanarius* and a decrease in *Dasychone* and *Salmacis* from January to June. It is presumed that these alterations in size are caused by variations in salinity.

4. In December-January preparations of *Dasychone*, fat—the only deutoplasmic inclusion—appears to be formed by the transformation of Golgi material.

5. In May-June fatty yolk alone has been observed in the eggs and this appears to be secreted by the dictyosomes.

6. The nucleoli and nucleolar buds are stained deep red in December-January while in May-June they are orange, of slightly varying shades.

7. In *Salmacis* also, there is a variation in the quantity of nutritive bodies, mitochondria and yolk in the two seasons.

8. An attempt has been made to show that the remarkable diversity of results obtained by workers on the same animal are due to variations in the environment: (1) seasonal, (2) geographical.

KEY TO LETTERING OF ALL TEXT-FIGURES.

c.s. Clear spaces left by fat vacuoles in Da Fano sections ; *f.* Fat vacuoles; *f.y.d.* Fatty yolk vacuoles ; *g.a.* Golgi apparatus ; *g.b.* Golgi batonettes ; *g.b.c.* Golgi batonettes occurring as a clump ; *m.* Mitochondria ; *n.* Nucleus ; *nl.* Nucleolus ; *nl.b.* Nucleolar buds.

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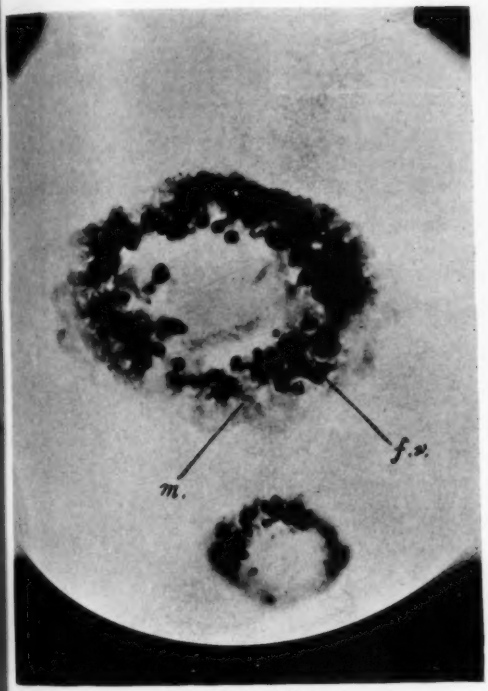
Addendum.

In some of the *Dasychone* material fixed in November 1935 we came across eggs in which fat and fatty yolk occur side by side. This intermediate condition gives additional proof of the existence of a seasonal variation.

Photomicrograph 1 shows an egg with fat droplets (*f. v.*) and granular mitochondria (*m*) taken from a preparation made in January-February 1933. In photomicrograph 2—a May-June preparation of the same year—can be seen fatty yolk vesicles (*f. y. d.*) and swollen mitochondria. The transitional stage can be seen in photomicrograph 3 (November 1935) where the mitochondria are becoming granular and where fat and fatty yolk occur side by side. Unfortunately no preparations were made in November 1933.

1



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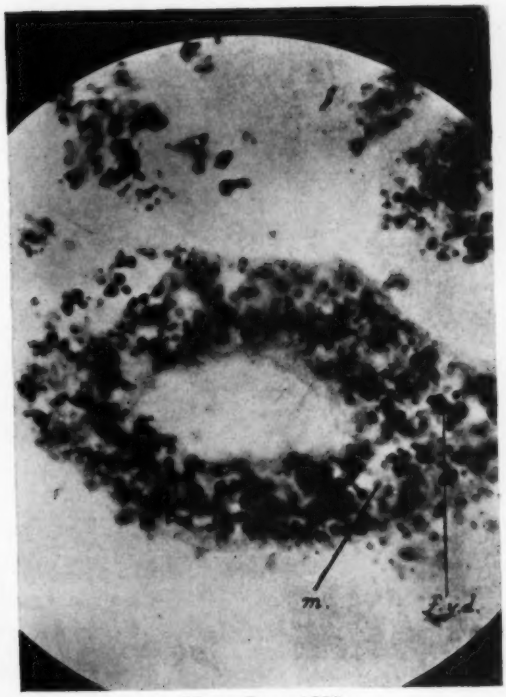

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A PRELIMINARY NOTE ON THE BIONOMICS AND ECONOMIC IMPORTANCE OF *MICROBRACON HEBETOR* SAY, A *BRACONID* NEW TO NORTH INDIA.

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(Communicated by Prof. C. R. Narayan Rao, M.A.)

Introduction.

The Hymenopterous parasite *Microbracon hebetor* Say was claimed as recorded for the first time from India by Glover (Norris Glover Aldis, 1935), after consultation with Dr. C. F. C. Beeson, Dr. Hem Singh Pruthi, Dr. T. V. Ramakrishna Ayyar and Mr. Afzal Hussain. Since this claim was published a record of *Habrobracon hebetor* (synonymous with *M. hebetor*) parasitic on *Corcyra cephalonica* in South India has come to our notice (Ayyar, 1934).

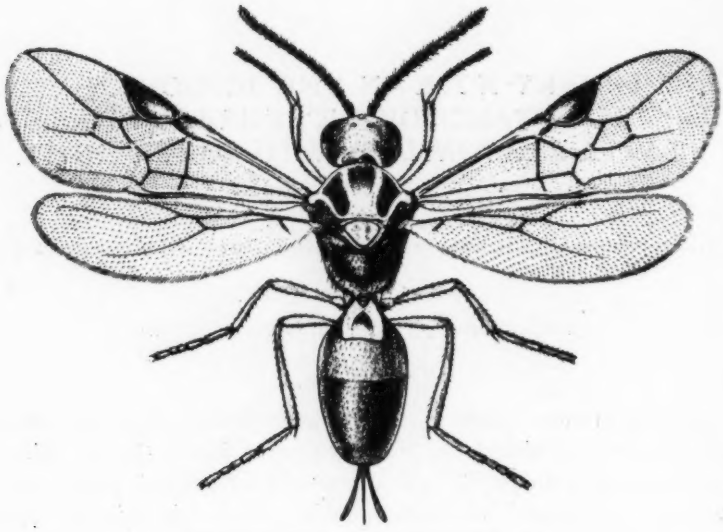
Acknowledgements.—The *Braconid* was identified by Dr. Ferrière by the courtesy of Sir Guy Marshall, Director of the Imperial Institute of Entomology.

We are indebted to Mrs. Dorothy Norris, Director of the Indian Lac Research Institute for her interest in this work and for advice in preparation of this paper, to Dr. C. F. C. Beeson, Forest Entomologist, Forest Research Institute, Dehra Dun, for advice and criticism and to Mr. Heber, Artist and Photographer at this Institute for preparation of the figure and plate.

There is an extensive literature on this species, particularly American. This paper makes no attempt to summarise previous work, its object being to give a preliminary description of the behaviour and economic importance of *M. hebetor* in India, with particular reference to its possible importance in lac cultivation.

Discovery of the Braconid *in India.*

Specimens of the *Braconid* were originally discovered on the glass window panes and walls of a room in the experimental Lac Factory at Namkum in July, where a number of samples of lac were stored prior to manufacture. They were taken while collecting *Microbracon greeni*, Ashm (syn. *Bracon tachardiæ* Cam.) from the same source.



2.5. m.m.

Microbracon hebetor, Say. Female Adult.

FIG. 1.

M. hebetor occurs in Ceylon. Mr. F. P. Jepson, Acting Entomologist, Peradeniya, addressed on the subject, kindly forwarded my letter to Mr. C. B. Redman King, Entomologist at the Tea Research Institute, Ceylon, to whom the senior author is indebted for the following information :—

Hosts attacked in Ceylon :—

Ephestia kühniella.
Ephestia cautella.
Sitotroga cerealella.
Corcyra cephalonica.

The Tortrix *Homona coffearea* was utilised as a host in one instance, and *E. elutella* and *Plodia interpunctella* are regarded as probable hosts. He records an instance of 44 eggs being laid by a single female, out of which 37 hatched. I am also indebted to him for the following life-history data :—

		Average	Number
Eggs ..	3—4 days	3·2 days	37 eggs
Larvæ ..	3—4 ,,	3·3 ,,	23 larvae
Pupae ..	10—12 ,,	11 ,,	18 pupae
Adults, Males	3—10 ,,	7·7 ,,	5 males
Females ..	11—12 ,,	11 ,,	6 females

at an average temperature 72°F. (maximum 80°F.).

Hosts of M. hebetor.

Host species in various parts of the world include such important pests as

Ephestia kühniella.
E. cautella.
E. elutella.
Sitotroga cerealella.
Corcyra cephalonica.
Plodia interpunctella.
Galleria mellonella.
Pyralis farinalis.

Pyrausta nubilalis and *Cydia* (*Carpocapsa*) *pomonella* are stated to be hosts, and Johnston (1927) has been investigating the possibility of transferring *M. hebetor* to *Platyedra gossypiella* in Egypt.

Of these host species *E. cautella, C. cephalonica, S. cerealella, G. mellonella, P. interpunctella* and *P. farinalis* occur in India as pests. *H. coffearea* has been recorded as a pest of tea and coffee in South India. The possibility of the transference of *M. hebetor* to *Platyedra gossypiella*, the pink boll worm of cotton, in India is under investigation. *M. hebetor* has already been induced to oviposit on *P. gossypiella* under laboratory conditions at Namkum.

Microbracon hebetor is therefore an extremely beneficial parasite.

Host species of M. hebetor *at Namkum.*

Microbracon hebetor adults were caged with the lac predators, *Eublemma amabilis, Holcocera pulverea* and *Eublemma scitula*. The *Braconid* was found to be an ectoparasite of these lac enemies.

E. amabilis and *H. pulverea* are the most serious lac enemies in India; they are cosmopolitan occurring in all lac growing districts including Assam and Burma, and are responsible for about 35% damage to the lac crops.

E. scitula larvæ are predatory on a number of injurious *Coccid* species; *E. scitula* has however been recorded as a pest of lac in South India. It is not an important enemy of lac in Northern India, it occurs sporadically at Namkum on lac in very small numbers. In the large number of samples received and examined at this Institute *E. scitula* has been encountered only in consignments of lac from Bijapur, Bombay; and in a sample of *Acacia Catechu* lac from Kashmir.

Microbracon hebetor is thus an extremely beneficial insect worth encouraging. It has the disadvantage of being parasitic on *E. scitula* which feed on injurious *Coccids*, which is, however, outweighed by its beneficial habits, particularly as *E. scitula* may itself at times be harmful owing to its habit of feeding on lac.

Conditions under which the host species live and under which parasitism occurs.

The larvæ of *E. amabilis* and *H. pulverea* (*cf.* Misra, Negi, Gupta, 1930, and Misra, Gupta, 1934) after hatching from the egg laid on or near the lac encrusted branches, bite their way into the encrustation and feed on the lac insects and lac tests from within. The whole of the larval and pupal life is passed within the encrustation in a gallery or tunnel lined with silk, excreta and particles of resin. It is while within this gallery that parasitism occurs. In immature lac the galleries approximate to the surface, the wall of the gallery being coincident with the outside of the encrustation, oviposition can occur readily in such places (Plate II, Fig. 9). In mature lac the wall of the galleries may be coincident with the surface of the encrustation in some or in many places; on the other hand, the gallery may lie well below the surface and be accessible only *via* the tubercular pores of the tests of the devoured lac insects; under these circumstances oviposition is less easily accomplished. This later condition is particularly common in lac grown on *Schleichera trijuga* and occurs even in immature lac on this host (Plate II, Figs. 1—7). In lac grown on hosts such as *Butea frondosa* and *Zizyphus jujuba*, the galleries usually approximate to the surface in immature lac and frequently do so in some places at least in mature lac also. Figures showing the galleries and domes of *E. amabilis* are given in Misra (1928) Plate XIII, Figs. 14, 26, 27; Plate XVIII and Fig. 29; and in Glover (1930) Plate IX and (1931) Fig. 22. Damage by *H. pulverea* is illustrated by Misra and Gupta (1934) Fig. 2 (*a*).

The larva of *E. scitula* after hatching from the egg, bites its way into the lac encrustation and feeds on *L. lacca* from within ; after the 2nd instar the larva comes to the outside and feeds covered by a 'dome' shaped shell of silk, and pieces of lac encrustation ; the larva crawls over the encrustation concealed by its dome or shell feeding. At this stage, parasitism occurs.

Technique of laboratory oviposition and breeding.

Battery jars 4" × 5", covered with fine mesh brass gauze covers, were used as oviposition cages ; a swab of cotton wool soaked in dilute sugar solution placed on a watch glass on the bottom of the cage, provided the food for the parasites.

Naked host larvæ were not offered to *M. hebetor* since *E. amabilis*, *H. pulverea* and *E. scitula* larvæ are never exposed under natural conditions. (Later observations, however, have shown that naked *E. amabilis* and *H. pulverea* larvæ are also parasitised by *M. hebetor*.) Moreover, Salt (1931) referring to species of the Genus *Microbracon* in his notes on *M. terebella* Wesmll. points out that although a wide range of hosts are parasitised, *Microbracon* species prefer hosts from which they are separated by an inert covering ; and that in over 90% of the records (Farnham House Laboratory) the parasite is separated from the host by a covering of inert material.

The method of presentation of *E. amabilis* described in the following paragraph was similar to that employed for breeding *Microbracon greeni*, which is ecto-parasitic on *Eublemma amabilis*, which was originally devised by Messrs. Negi and Gupta of the Entomological Department of this Institute.

Sticks of lac as shown in Plate II, Fig. 1 were collected and the encrustation (*e*) broken from the stick (*s*) by coolies, such pieces are shown in Figs. 2—7 ; Fig. 2 shows the external surface ; attack by *E. amabilis* is not apparent ; Figs. 3—7 illustrate the internal surface ; Figs. 3 and 5 illustrate empty galleries ; Fig. 4 a gallery containing a larva (1). Pieces of lac in which galleries containing living larvæ were found, were allowed to lie overnight in glass dishes, during which time the larvæ spun a covering over themselves of silk and excreta ; successive stages in the formation of such a covering (*d*) are shown in Figs. 4, 7 and 6. Generally any *E. amabilis* larvæ placed in a gallery will behave in this manner ; thus the pieces of lac containing the gallery could be used over and over again and the collection work only entailed the obtaining of larvæ. Larvæ in the 10th instar nearing pupation often failed to spin ; in these cases they were covered with fine tissue paper held in position by 'plasticene'.

A number usually 4—6 such pieces were then attached to a short piece of stick by means of plasticene with the web surface upwards Plate II, Fig. 8.

This stick was then fastened to the inside wall of the battery jar by a blob of 'plasticene', the stick projecting downwards into the cage.

This is referred to as the 'dome' method of presentation.

An alternative method of presentation of *E. amabilis* and one which was used in the case of *H. pulverea*, was to collect a number of short pieces of lac infected by either predator and attach them, after examining for prior parasitism, to a piece of stick by means of 'plasticene' and introduce this in the manner already described into the oviposition cage. This is referred to as the 'Infected stick' method of presentation.

A third method which gave satisfactory results, and was equally suitable for either *E. amabilis* or *H. pulverea* was as follows: Short strips of wood about $3\frac{1}{2}''$ long and $\frac{3}{4}'' \times \frac{3}{4}''$ were taken and a groove about $\frac{1}{4}''$ deep by $\frac{1}{4}''$ wide was cut in one face extending to within about $\frac{1}{4}''$ from each end of the stick. The groove was divided into about 5—6 chambers by small cork partitions. Into each chamber a host larva was introduced, the groove containing the larvæ was then covered over with a strip of tissue paper held down with 'plasticene'. These sticks were introduced into the cages. In some instances the larvae spun webs in the chambers, and in these instances the tissue was removed before introducing the stick into the oviposition cage. This is referred to as the 'Artificial stick' method of presentation.

E. scitula larvæ were introduced on short sticks of lac on which they were found or on which they had been placed to feed.

All the above methods resulted in satisfactory oviposition.

The sticks were removed daily and examination made of the larvæ by cutting open the web or removing the tissue. Parasite eggs and larvæ were removed with their host to a glass block in which the remainder of the life-history could be followed. A new stick was then introduced into the oviposition cage.

These methods ensured the ready accessibility of the host, and allowed examination of the hosts for disease or previous parasite attack prior to their introduction into the oviposition cages. They were in addition simple and convenient in use.

Mating.—Mating was not observed.

Oviposition.

(1) *Behaviour.*—Females alight on the sticks introduced into the cages and appear to locate the host larvæ by feeling with the antennæ which probably contain an olfactory sense as suggested by Myers (1927) in the

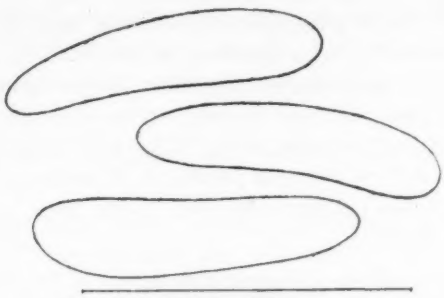


FIG. 2.

case of *Alysia manducator*. Having located a host larva, the female behaves in one of two ways apparently at random :—

Direct Oviposition.—The female bites her way through the inert covering where this is a thick web or tissue paper, or wriggles her way through it if it is a loose web as is often the case with *H. pulverea*. She finds her way to the host larva which she stings either actually standing on it, or standing close to it bending the abdomen downwards and forwards so that the ovipositor projects to the front between her legs. The female is not easily disturbed ; eggs are then laid on or close to the host larva. Females were observed ovipositing direct on *E. amabilis* and *H. pulverea* both in 'domes', 'infected sticks', and 'artificial sticks'.

Indirect Oviposition.—The female having located the web or gallery of a host, examines the surface with her antennæ, then raising the body and bending the abdomen downwards so that the ovipositor projects downwards and slightly forwards, she feels the surface with the palp-like organs of the ovipositor sheath which are believed to be tactile (*cf.* Myers, 1927) ; the ovipositor is then forced through the covering of the gallery repeatedly, apparently a searching action. When the host is found, it is stung and eggs laid on or close to it, through the inert covering. Indirect oviposition occurred on *E. amabilis* and *H. pulverea* through webs and tissue and on *E. scitula* through the dome-like shell.

During stinging a neuro-toxin appears to be injected, paralysis occurs and movements are arrested, but the heart continues to beat slowly for some time. Sting marks may or may not be apparent, when present they take the form of irregular light or dark brown patches. As many as 6—7 were observed in some instances.

In the case of direct oviposition the female may be observed feeding at the punctures made by the ovipositor, as described by Myers (1929). In the case of indirect oviposition, feeding tubes are constructed from the host body to the inert covering, and feeding occurs through them by suction, exactly as described by Trouvelot (1921, 1923, 1924) in the case of *M.* (*Habrobracon*) *johannseni* and by Génieys (1925) for *Microbracon brevicornis*. The number of feeding tubes per host observed varied from 1—3. They are not observed in every instance.

Eggs may be deposited on almost any region of the host and frequently free in the gallery close to it. In the case of *E. scitula*, eggs were found in each instance below the larva on the ventral tissue floor of the dome-like shell. Oviposition occurs at the junction of the shell and the ventral tissue wall.

Several females were at times observed ovipositing simultaneously on the same host, and also females in certain instances oviposited on the same host as many as three times over a period of 2—3 days. This explains the high figures for eggs per host in some of the multiple cages. This may be defined as semi-destructive superparasitism as although a considerable number of eggs and larvæ fail to survive, a number usually completed the life-cycle.

In one cage *E. amabilis* larvæ kept at reduced temperature in a refrigerator (recorded, refrigerator 12°C.—27°C. : room temperature 26°C.—30°C.) for 8 days to delay pupation were offered as hosts, in September. In two instances oviposition occurred ; 4 eggs were laid on 1 larva, and 6 eggs (1 damaged) on another, 2 days after removal from the incubator. In each case the host dried up 2 days after oviposition. One larva and 3 unhatched eggs still surviving were transferred to a fresh unstung *E. amabilis* larva whose head was crushed to restrict movement. The eggs hatched ; the host, however, began to dry up ; 1 larva reached the final instar but did not survive.

Oviposition and longevity in the laboratory.

Both males and females of *M. hebetor* are capable of squeezing through very small spaces, and escapes were common from the oviposition cages. Of the 44 males caged 47·7% escaped after an average of 3 days. Of the 53 females caged 49% escaped after 1—19 average 4·5 days.

60·3% females failed to lay eggs ; 32% escaped, 18·9% died and 9·4% were crushed, stuck to sugar swabs or were killed and set. The remaining 39·7% oviposited ; 17% escaped, 1·9% were damaged and 20·8% reached the end of the oviposition period and died natural deaths.

Unfertilised females oviposit, the eggs however invariably give rise to males only. Of 15 unfertilised females 4 escaped, 4 were set or stuck to sugar swabs and 2 died, without ovipositing; the remaining 5 laid eggs.

Host larvæ may be stung and the parasites may feed on them without any eggs being laid.

In the above, adults caught in the Factory of the original generation are omitted as exact counts could not be made.

In most cases host larvæ proffered were in the final instar. In the case of *E. amabilis* eggs also were laid on larvæ in the 8th and 9th instars and in two cases on early prepupæ.

The number of eggs laid per host larva is recorded in Table I.

TABLE I.

Number of eggs laid per host larva by M. hebetor.

Host	Single fertilised female	Pair fertilised females	Several fertilised females	Single unfertilised female	Several unfertilised females
E. amabilis	1—11 av. 6 (on 12)	2—18 av. 6 (on 18)	8—43 av. 16 (on 9)	2—9 av. 5 (on 5)	2—3 (on 2)
H. pulverea	1—12 av. 4 (on 10)	2—7 (on 3)	1—17 av. 7 (on 41)	1—9 (on 2)	1—12 av. 6 (on 7)
E. scitula	..	3—9 (on 3)	..	..	..

The average number of eggs laid per *E. amabilis* larva was 8, per *H. pulverea* larva 6 and per *E. scitula* 5. The average number of eggs per all hosts was 7. *E. scitula* larvæ were only offered as hosts in one case. *E. amabilis* seems to be the most favoured host.

In the case of *E. amabilis* 42% parasitised larvæ failed to give rise to adults at all, from the remainder 1—7 adults were bred, the average being 2·6. No adults were bred from 61% of the parasitised *H. pulverea* larvæ; from remainder 1—8 average 2·5 adults were bred. In two of the three cases in which *E. scitula* was utilised as host 1 adult was bred, in the third no adults were bred. *E. amabilis* is again the more satisfactory host.

The number of eggs laid per female fertilised and unfertilised are recorded in Table II.

TABLE II.

Number of eggs laid per female M. hebetor.

Generation	No. of eggs laid	No. of females caged	No. of females escaped	Average No. of eggs per female
	Fertilised			
Caught in Factory ..	433	10(?)	..	43·5
1st ..	1	1	..	1·0
	116	4	3	29·0
2nd ..	19	1	..	33·5
	48	1	..	
	73 (21 prior to fertilisation)	7	4+1 damaged	10·0
3rd ..	8	1	..	8·0
	11	1	1	10·0
	Unfertilised			
1st ..	36	4	1	9·0
4th ..	26	1	..	26·0

Fertilised females which after the oviposition period died naturally, laid an average of 36·5 eggs per female. Maximum and minimum numbers were 48 and 1. All females which oviposited regardless of escapes, laid an average of 27·3 eggs per female. An unfertilised female which completed the oviposition period and died a natural death laid 26 eggs. The average number of eggs per unfertilised female regardless of escapes was 12.4.

Oviposition and longevity data for fertilised and unfertilised females are recorded in Table III.

The average longevity of fertilised females which oviposited and died natural deaths was 4—12 average 7 days (No. of females 7). The average longevity of unfertilised females which oviposited and died natural deaths was 11—30 average 17 days (No. of females 4).

In a few instances females were seen apparently entangled in the webs of *H. pulverea* larvæ, on which they had oviposited, some managed to disentangle themselves, others struggled violently for some minutes and finally died.

TABLE III.

Oviposition and Longevity Data (M. hebetor).

Gen.	Dates	Pre-oviposition	Oviposition	Post-oviposition	Total longevity	Number of females
			Fertilised females			
Caught in Factory	28-7-34 to 12-8-34	1 day	15 days	0 days	16 days	10 females in cage
1st Gen.	10-8-34 to 1-9-34	2-5 days	1-10 days	0-1 days	5-12 days	3 single females 1 pair females } 3 escaped
2nd Gen.	1-9-34 to 24-9-34	1 & 2 days 2-3 days	3 & 6 days 2-14 days	0 & 2 days 3-6 days	4 & 10 days 2-19 days	2 single females 7 females, 4 escaped, 1 crushed
3rd Gen.	16-9-34	4 & 6 days	2 & 7 days	1 & 1 days	7 & 14 days	2 single females, 1 escaped
			Unfertilised females			
1st Gen.	17-8-34 to 30-8-34	8 days	6 days	0 days	9-14 days	4 females in cage, 1 escaped
4th Gen.	6-10-34 to 5-11-34	12 days	15 days	3 days	30 days	1 female

The average longevities of males are recorded in Table IV.

TABLE IV.

Longevity of males (M. hebetor).

Generation	No. of males	Longevity
1st Gen. ..	10	1—12 average 4·5 days
2nd Gen. ..	6	1—10 average 6 days
3rd Gen. ..	6	1—7 average 3 days

The total longevities of males were 1—12 average 3 days.

Fertilised females laid eggs which gave rise to males and females in approximately equal numbers (44·6% males, 55·4% females).

Life-History.

Life-history data are given in Table V. It will be seen that the egg stage varied from 1—2 days, the larval stage from 5—9 days, the pupal

TABLE V.

Life-History Data (M. hebetor).

Generation	Egg period	Egg Mortality	Larval periods		Mortality		Pupal periods			Total cycle		No. and sex	Percentage adults bred	Month	Remarks
			Before spinning	Within cocoon	Total	Larval	Male	Female	Pupal Mortality	Male	Female				
1st ..	1 day	32·4%	2·5 days	3 days	6 days	76%	4 days	4 days	20·6%	11 days	11 days	21 ♂ ♂ 33 ♀ ♀	12·5%	Aug.	
2nd ..	1 day	16·7%	2 days	3 days	5 days	64·2%	4 days	3 days	20·9%	10 days	10 days	19 ♂ ♂ 15 ♀ ♀	22·4%	Aug. Sept.	
3rd ..	1 day	36·3%	2 days	3 days	6 days	71·4%	5 days	4 days	9·1%	12 days	11 days	12 ♂ ♂ 8 ♀ ♀	16·1%	Sept.	
4th ..	1 day	39·0%	3 days	3 days	6 days	90·9%	—	3 days	0%	—	10 days	1 ♀	5·6%	Sept. Oct.	Single ♀ only bred.
5th ..	2 days	19·2%	4 days	5 days	9 days	50%	7 days	—	10%	18 days	—	9 ♂ ♂	34·6%	Oct. Nov.	All laid by single unfertilised ♀

stage from 3—7 days and the total cycle from 10—18 days. Mortality was high throughout particularly in the larval stage where it averaged 72%, the average mortality in the egg stage was 30% and in the pupal 18%. The percentage adults bred from the eggs laid was only 15·7%. Among the larvæ 49% failed to complete cocoons, the failure to do is believed to be due to lack of support in the concave glass blocks used for breeding where only a single larva was kept or where the host remains were removed, as suggested by Glover (1934) for *Microbracon greeni*. Larvæ which failed to spin cocoons are capable of pupating and giving rise to adults.

During examination of all stages damage during manipulation was only 1·2%; where damage occurred, it was usually while attempting to disentangle eggs or larvæ from *H. pulverea* webs.

Larval mortality included certain instances of cannibalism which was however rare. A common cause of mortality was larvæ failing to attach at once or hatching straying away and drying up. In rare instances parasitised host larvæ were insufficiently paralysed, recovered and became active, shaking off the parasite eggs or larvæ.

For comparison with oviposition and life-history results (Tables IV and V), the field temperature and humidity conditions during this time are recorded in Table VI.

The strain died out due to one female only being bred in the 4th generation; being unfertilised, she gave rise only to males; she survived sufficiently long for the first male in her progeny to be introduced into the oviposition cage with her, but died on the day of his introduction.

TABLE VI.

Field temperature and humidity conditions during oviposition and life-history for M. hebetor.

	July	August	September	October	November
Max. Temp. ..	90°F.	87°F.	91°F.	91°F.	86°F.
Min. Temp. ..	72°F.	71°F.	69°F.	55°F.	41°F.
Av. Max. ..	86·2 °F.	84·5°F.	85·8°F.	84·4°F.	79·2°F.
Av. Min. ..	74·11°F.	73·1°F.	73·1°F.	64·2°F.	53·4°F.
Av. Mean ..	80·2°F.	78·8°F.	79·5°F.	74·3°F.	66·3°F.
Humidity ..	85%	84·8%	82%	61%	48%

Discussion.

M. hebetor, although discovered at Namkum in the Lac factory where it would appear to have emerged from stored lac, is not regularly obtained from lac. It seems probable since specimens have not previously been obtained at this Institute (or have been so only rarely) that it has a host in some *Lepidopterous* larva, possibly a stem borer and that this instance of its being obtained from lac was an abnormality in behaviour. This might account for the failure in some instances of females to oviposit on lac predators, and for the high mortality of parasite larvæ when they were utilised as hosts. *M. hebetor* is believed to be rare in North India.

The fact that *M. hebetor* will oviposit on and can be bred from both major lac predators *E. amabilis* and *H. pulverea*, although neither species is its normal host, suggests it as an ideal parasite for acclimatisation to lac predators and for introduction in lac growing areas. All its recorded hosts in Ceylon and elsewhere are pests of varying importance several of which occur in India; thus its introduction is likely to be beneficial in other directions. Its parasitism of *E. scitula* which feeds on injurious *Coccids* is a disadvantage of minor importance; this latter species also occasionally occur as a lac predator.

The high percentage of escapes may be expected to be materially reduced by improvements in breeding technique. The method described also could be considerably simplified, but was utilised in order to obtain individual data. The high percentage mortality among larvae will probably be reduced when *M. hebetor* has been acclimatised to its new hosts. The fact that *E. kühniella* is utilised as a host in Ceylon suggests a useful easily obtained reserve laboratory host among the *Ephestia sp.*

The 'escapes' occurred in the laboratory room in which the breeding work was carried out; they were unable to get out and died in the room, and hence had no chance to found a colony at Namkum in the field. As no further specimens have been collected at Namkum, arrangements were made with Mr. C. B. Redman King, Entomologist at the Tea Research Institute, Ceylon, to have specimens sent per post to Namkum in order to start further breeding experiments. I should like to record my thanks to him for his assistance.

On March 25th, 1933, about 40 cocoons of *M. hebetor* in the crevices of a small piece of corrugated paper were despatched from the Tea Research Institute, Ceylon. Of the cocoons sent, the oldest (in cold storage) was 10 days.

The consignment arrived at Namkum on April 3rd, 1935. On opening 4 males and 5 females were found to have emerged; two further males emerged on April 4th. No further emergence occurred. A count was made of the number of cocoons which was found to be 38. Percentage emergence was therefore only 28·9%, of the remainder, one contained a dead male and one a dead female, 3 contained recognisable dead prepupæ, and 32 contained dead larvæ.

The living adults were caged for oviposition, which it is hoped to describe in another paper which will include description of the method in which *M. hebetor* was bred on *Ephestia kühniella* larvæ also obtained from Ceylon.

Summary.

M. hebetor Say is recorded for the first time from North India and notes are given of its behaviour and hosts in Ceylon.

In India its usual host has not been discovered, it was discovered in a room where lac was stored, and was bred in the Laboratory as an ecto-parasite of *Eublemma amabilis* and *Holcocera pulverea*, both serious enemies of the lac cultivator. *M. hebetor* is believed to be rare in North India.

A preliminary detailed description of its bionomics, breeding and life-history is given: the hosts used were *E. amabilis*, *H. pulverea* and *E. scitula*, the latter although occasionally a lac predator is a beneficial rather than harmful insect on account of its larval habit of feeding on injurious *Coccids*. *M. hebetor* was induced to oviposit on *Platyedra gossypiella* larvæ under laboratory conditions at Namkum.

The hosts of the *Braconid* in other parts of the world are all pests of varying importance, largely of stored products. Many of these pests occur in India.

The percentages of escapes, failures to oviposit and mortality among the life-history stages are rather high; these, it is considered, will be reduced with improved breeding technique and with acclimatisation to *E. amabilis* and *H. pulverea* as hosts. The *Braconid* is considered to be a potentially ideal parasite for acclimatisation and introduction in lac-growing areas.

As no further specimens have been collected or obtained here, arrangements were made to obtain a consignment from Ceylon for further investigation; the consignment arrived safely giving a percentage of only 28·9% adults.

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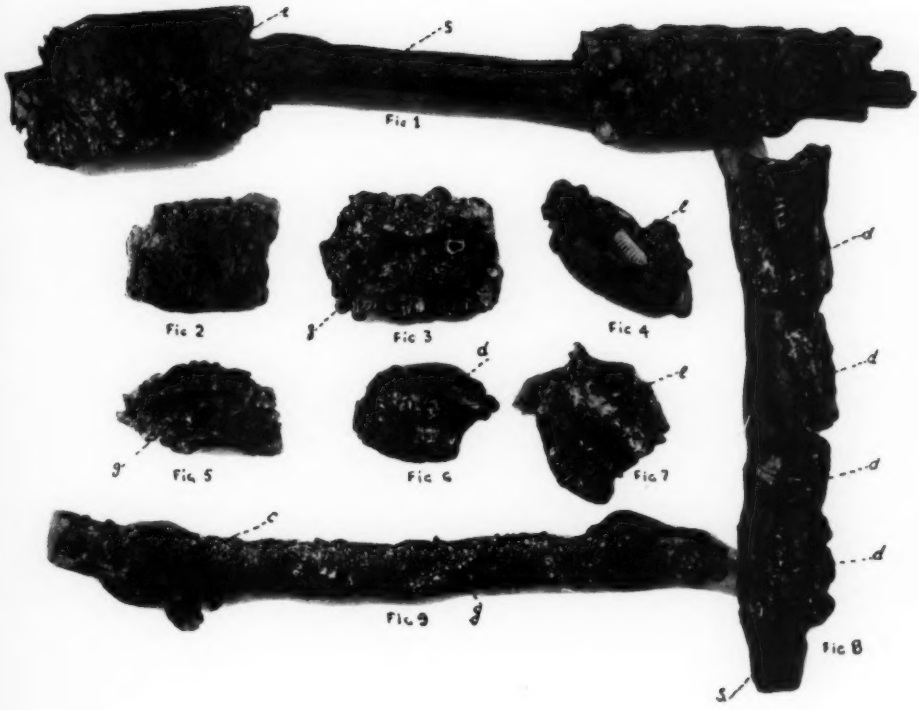
Fig 1
Fig 2
Fig 3
Fig 4
Fig 5
Fig 6
Fig 7
Fig 8
Fig 9

EXPLANATION OF PLATE.

(*Natural Size.*)

FIG. 1.—Stick of mature *L. lacca* grown on *Schleichera trijuga*; pieces of the encrustation (*e*) have been broken from the stick (*s*). Such pieces are illustrated in figures 2-7.

FIG. 2.—External surface.

FIG. 3.—Internal surface, showing empty *E. amabilis* gallery (*g*).

FIG. 4.—Larva of *E. amabilis* (*l*) in gallery.

FIG. 5.—Empty gallery (*g*).

FIG. 6.—Gallery in which the larva has constructed a "dome" (*d*).

FIG. 7.—"Dome" opened to show the larva (*l*) inside.

FIG. 8.—A "pseudo" stick ready for introduction into an oviposition cage; note "domes" (*d*).

FIG. 9.—Young female cells (*c*) of *L. lacca* shortly after male emergence on *Butea frondosa* showing larval gallery (*g*) of *E. amabilis*.

PLASMODIUM NARAYANI N.SP., PARASITE OF THE FISH OTTER *LUTRA LUTRA*.

By Col. I. Froilano de Mello
and
Emerciano Dias.
(Nova-Gôa.)

Received February 3, 1936.

A fish otter shot at Nagoa by Mr. Thomàs Osório gave us the opportunity of studying a plasmodid of this animal not yet recorded in the protozoological literature. The skin was kindly identified by Mr. Prater, Curator, Bombay Natural History Society and to him our best thanks are due.

The parasite is very scanty, and has been found only in the blood and lung smears. The liver, kidney, spleen and bone marrow did not show any peculiarity worthy of mention.

All the stages of the schizogonic cycle of this plasmodid are very similar to those of a human plasmodium. The young schizont has an annular form, with its protoplasm either very thin as in the initial rings of human malaria or more or less compact. Some red cells are parasitised by two schizonts. The parasite was not seen in living condition, but must be strongly ameboid as many aberrant forms are found, fusiform, in crescent moon, or triangular as figured in the plate. The young schizont occupies one-sixth to one-fifth of the red cells size and shows seldom one or two granules of coffee-brown, almost black pigment. We have not found enough specimens of adult schizonts or trophozoites to describe fully their morphology. It seems that they are rather round, but in some cases it is not possible to state definitely whether such forms are really trophozoites or young gametocytes. In a further stage of development when the chromatin begins to divide (prerosette), the trophozoite is round and many granules of pigment, some of them rod-shaped, fill the protoplasm of the parasite. The chromatin is lodged in a clear, vacuolated zone, in contrast with the remaining part of the protoplasm which is more compact.

Very rare rosettes of multiplication were found in the lung smears, with 9 to 11 merozoites.

The gametocytes are oval, with a compact chromatic oval nucleus situated in the middle and some granules and rods of pigment irregularly

situated in the surrounding area. We believe that we did not find male gametocytes, and the figures in the plate represent but female specimens.

The red cell is sometimes dehemoglobinised, somewhat enlarged, but does not show any granules or dots of any kind.

No blood parasite has been recorded in *Lutra lutra*. We consider therefore our plasmodid is a n. sp. with which we associate the name of Prof. C. R. Narayan Rao, of the Central College, Bangalore, as a homage to his distinguished career and his well-known activities in the Indian Academy of Sciences.

The Coloured Plate will appear in the next issue.

THE INHERITANCE OF CHARACTERS IN THE GROUNDNUT *ARACHIS HYPOGAEA*.*

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Received September 10, 1935.

Introduction.

The groundnut is the most important oil-seed crop of the Madras Presidency with an annual acreage of about three million acres. The genetics of the crop has not received the attention that the importance of the crop would warrant. Stock (1910), Badami (1928), Stokes and Hull (1930), and Hayes (1933) have determined the inheritance of some of the characters. At the Agricultural Research Station, Palakuppam, South Arcot District, the breeding of improved types has been in progress during the last five years. Some of the characters, the inheritance of which has been studied, are of considerable economic importance. The inheritance of the following characters is reported in this paper :—

1. Chlorophyll deficiency.
2. Abnormality.
3. Growth habit.
4. Branching.
5. Duration.
6. Pubescence.
7. Testa colours.
8. Anthocyanin pigment.

Material.

A collection of about a hundred "varieties" of groundnut has afforded the authors a wide range of material for breeding work and also for the study of inheritance of characters. The characters studied have been described separately under each head. Of the varieties used in the various crosses, mention must be made of H. G. 1 (Hebbal groundnut) which is a bunch extract isolated by Dr. Badami from the progenies of a cross between a spreading

* Contribution No. 8 from the Oil-Seeds Section, Madras Agricultural Department.

and a bunch variety. Other varieties used in the work are pure lines isolated from the different varieties growing in the station.

There are two main seasons for growing the groundnut, *viz.*, rainfed and irrigated. Irrigated groundnuts are grown during February to June period, but this summer crop is very limited in area. The material for the crosses was grown mostly in the rainfed season—July-August to December-January. Advantage could not be taken of the irrigated season since the crop in that season was subject on the farm to the attack of diseases and pests. The material presented in the paper records the observations made from 1931 onwards.

Crossing Technique.

Flowering in the groundnut starts from the third week after sowing. The short duration bunch varieties begin to flower about two or three days earlier than the long duration spreading varieties. Flower buds appear in leaf axils singly or in clusters of two to five. The maximum number of flowers appearing in any one leaf axil never exceeded five. The flowers when in clusters develop and open in acropetal succession. The deep-seated and congested cluster of the flower buds are protected by closely packed bracts. This makes the removal of flowers not wanted, difficult, and thus a fairly large percentage of selfed seeds may be left in crossed plants. The flowers bloom between six to eight in the morning and the blooming of flowers occurs mostly at random. The flowering period lasts for about 45-60 days in the bunch varieties and 60-70 days in the spreading varieties. The flowering is very much affected by the distribution of rainfall.

Stokes and Hull (1930) have described the crossing technique in the groundnut plant. At the Agricultural Research Station, Palakuppam, the procedure described below was found adequate for purposes of crossing. Emasculation is done by opening the flower buds between 5 P.M. and 6 P.M., about twelve hours before the time of blooming. The flower bud is gently held by the left hand and with the right, the standard, wing and keel petals are gently opened by a pair of fine forceps. Then the flower is emasculated, the tips of filaments are examined with a magnifying lens so as to ensure the complete removal of the anthers. After the anthers are removed, the petals are gradually manipulated to their original position for protecting and covering the stigma. Care has to be taken to see that the calyx tube or any of the parts of the flower are not twisted or disjointed in the operation. Artificial pollination of the emasculated flower is carried out the following day between 7 A.M. and 8 A.M., when the anthesis of flowers normally occurs. The success in crossing depends on the carefulness of the workers. From 10 to 30 per cent. of the artificially pollinated flowers produce pods. Natural

crossing must be extremely rare since the authors have not come across a single instance during the course of work extending over five years. Bagging with muslin bags is not considered necessary to prevent natural crossing. Bagging, however, may be a valuable adjunct where protection of flowers from beetles and of pods from birds is necessary. It has been found convenient to grow the plants for crossing in flower pots about 2 feet in diameter and 18 inches high. The plants are then at a convenient height for working and there is no difficulty felt during the rains. The crossed flower is marked by means of a particular coloured thread. When the flower fades and as soon as the gynophore is first seen, in four or five days, the thread has to be transferred to the gynophore.

TABLE I.

Inheritance of chlorophyll deficiency.

Cross number	Parents	F_1 phenotype	F_2 phenotype	
	$G_1G_1g_2g_2$ $g_1g_1G_2G_2$		Green	Albino
25	Philippine white (spreading) × Corientes-3 (bunch) ..	Green	1468	60
25 (*a*)	Reciprocal	Green	228	15
26 (*a*)	Philippine white (spreading) × Small Japan (bunch) ..	Green	487	40
28	Philippine white × Gudiyattam (bunch) ..	Green	1097	57
34	Saloum (spreading) × Gudiyattam (bunch)	Green	543	26
35	Madagascar × Gudiyattam (bunch)	Green	352	18
35 (*a*)	Reciprocal	Green	380	22
36 (*a*)	H. G. 1 (bunch) × Spanish 10 (bunch)	Green	333	19
	Observed		4888	257
	Expected on 15 : 1 ratio ..		4823	322
	$\frac{\text{Dev.}}{\text{S. E.}} = 3 \cdot 76$			

1. *Chlorophyll deficiency.*—The occurrence of the chlorophyll deficiency has been recorded in many crops, but the outstanding work has been in *Zea mays*, where over seventy genes affecting the chlorophyll have been recorded. Since albino seedlings cannot carry on photosynthesis, they do not survive to produce seeds, and therefore the study of albinism is made through the crosses between the two green types. The albino seedlings usually appear in the second generation of crosses between the bunch and the spreading types. In no instance did the authors come across albino plants in the F_2s of crosses between spreading varieties. In the crosses among the bunch varieties also, no albinos were obtained except when H.G.1 was introduced as one of the parents. In the above table the segregation of the F_2 population is shown.

The deviation of the observed numbers from the expected numbers on the basis of 15 : 1 ratio is significant. Further evidence for the bifactorial difference hypothesis is furnished from the behaviour of F_2 in the succeeding F_3 generation. If the hypothesis is correct 15 : 1 and 3 : 1 ratios must be obtained in F_3. From Table II it may be concluded that albinism in the groundnut is digenic :—

TABLE II.

F_3 segregation for albinism.

F_2 genotypes	F_3 phenotypes	
	Green	Albino
$G_1g_1G_2g_2$—total of 56 families		
Observed	1754	110
Calculated 15 : 1 ..	1748	116
$\frac{\text{Dev.}}{\text{S. E.}} = 0 \cdot 58$		
$G_1g_1g_2g_2$ and $g_1g_1G_2g_2$-total of 86 families		
Observed	1764	536
Calculated 3 : 1 ..	1725	575
$\frac{\text{Dev.}}{\text{S. E.}} = 1 \cdot 87$		

Badami (1928) as reported by Hunter and Leake (1933) has concluded that the triple recessive is the albino. The authors are, however, able to locate only two factors for albinism. It is possible that Badami was working with different material.

The ratio of 15 : 1 is obtained through the interaction of two duplicate factors each one of which produces identical effect. It has been previously mentioned that it was not ordinarily possible to obtain albino plants from among the crosses in which only bunch parents were used, and that generally crosses between the bunch and the spreading varieties yielded albino plants in F_2. The genetic constitution of the bunch and the spreading varieties must therefore be different. Let G_1 and G_2 represent duplicate genes which produce chlorophyll. The constitution of the spreading varieties is therefore $G_1G_1g_2g_2$ and that of the bunch varieties $g_1g_1G_2G_2$. The only instance where a cross 36(*a*) between two bunch parents yielded albinos in F_2 was when H. G. 1 was crossed with Spanish 10. This peculiar behaviour of H.G.1 is understood when it is pointed out that H.G.1 was extracted from a cross between a bunch and a spreading type. The genetic constitution of H.G.1 for chlorophyll should be similar ($G_1G_1g_2g_2$) to that of the other spreading varieties.

2. *Abnormality.*—The abnormal plants referred here were dwarf and stunted in growth having congested nodes and crumpled leaves. The plants showed normal branching but some of the secondary and tertiary branches developed fully and showed a highly congested condition of the nodes. The leaves were reduced in size and were wrinkled. These plants developed a few flowers which were mostly sterile. The pollen grains were empty and the stigmas were undeveloped. The plants were found to be free from insect pests or attack of any disease. Three plants out of 85 gave two small pods. This type of plants was obtained in the F_2 of the crosses in which Corientes-3 was crossed with the spreading varieties.

The F_2 plants showed a ratio of 15 normal to 1 abnormal suggesting the coming together of two factors from each of the two parents. The genetic constitution of Corientes-3 may be assumed as $N_1N_1n_2n_2$ and that of the other parent as $n_1n_1N_2N_2$. Hayes (1933) mentions sterility caused by a two factor difference segregating in F_2 in 15 : 1 ratio. It is possible that the abnormal plant mentioned here and his sterile plant are identical. Unfortunately he has not given a detailed description of his plants.

When Small Japan and Gudiyattam Bunch, the two bunch varieties are crossed with the spreading varieties, no abnormal plants are found in F_2. This indicates that these varieties are genotypically either $N_1N_1n_2n_2$ or $N_1N_1N_2N_2$. But when either of these two bunch varieties is crossed with

TABLE III.

Inheritance of abnormality.

Parents	F_1 Phenotypes	F_2 Phenotypes	
$n_1n_1N_2N_2$ $N_1N_1n_2n_2$		Normal	Abnormal
Philippine white × Corientes–3 ..	Normal	1332	73
Saloum × Corientes–3	Normal	237	12
Observed		1569	85
Expected on 15 : 1 ratio ..		1551	103
$\frac{\text{Dev.}}{\text{S. E.}} = 1 \cdot 84$			

Corientes-3, no abnormal plants are obtained in F_2. It may, therefore, be concluded that the Small Japan and Gudiyattam Bunch have in their genetic make up $N_1N_1N_2N_2$.

3. *Growth habit.*—The habit of growth of the plant influences considerably the agronomic practices. The optimum spacing and the mode of harvest in groundnut largely depend upon the habit of growth. The commonly cultivated variety may be resolved according to the habit into two groups, *viz.*, spreading and bunch. In the spreading varieties, the main axis grows erect, the two pairs of primary branches which take off from the main axis almost at right angles, grow along the ground and give a spreading or procumbent appearance to the plant. A few internodes, however, towards the growing top show a tendency to grow obliquely. In the bunch type the main axis grows erect and the primary branches which are usually four in number grow obliquely from the base of the main axis.

Seven crosses were made between the bunch and the spreading varieties, the F_1 plants in all of them showed complete dominance of the spreading habit, the F_2 population segregated mainly into the bunch and the spreading groups. The bunch habit in F_2 plants varied from typically erect to the ordinary bunch type, while the plants in the spreading group varied from the ordinary spreading to the trailing plants with completely prostrate branches. Repeated attempts to further classify the bunch and spreading groups at different stages of the growth of the plant did not give any intelligible ratios. This variation may be due to the presence of minor modifying factors or due

to the differential effect of the factors in the homozygous or heterozygous condition. We have yet no evidence for either of these two assumptions. The counts obtained in the F_2 are set down below :—

TABLE IV.

Inheritance of habit.

Cross number	Parents	F_1 phenotypes	F_2 phenotypes	
	$S_1 S_1 S_2 S_2$ $s_1 s_1 S_2 S_2$		Spreading	Bunch
25 (*a*)	Philippine white × Corientes–3	Spreading	162	49
25	Philippine white × Corientes–3	,,	1019	334
28	Philippine white × Gudiyattam (bunch)	,,	797	245
28 (*a*)	Philippine white × Gudiyattam (bunch)	,,	181	64
30 (*a*)	Saloum × Corientes–3 ..	,,	36	11
32	Saloum × Small Japan ..	,,	376	124
34	Saloum × Gudiyattam (bunch)	,,	250	87
35	Madagascar × Gudiyattam (bunch) ..	,,	271	89
26 (*a*)	Philippine white × Small Japan	,,	368	111
	F_2 hybrids		692	225
	Observed		4152	1339
	Expected on 3 : 1 ratio ..		4119	1372
	P between 0·2 and 0·3 x^2/m		0·264	0·879
	Expected on 13 : 3 ratio ..		4461	1030
	P less than 0·01 x^2/m ..		2·162	9·270

It is seen from the above figures that the bunch type is recessive. That the spreading type is probably the result of two complementary factors is evident from the data of another cross mentioned below. H.G.1 bunch variety when crossed with Spanish-10, another bunch variety, gave an F_1

spreading type and in the F_2 the spreading and the bunch types occurred in the ratio of 9 : 7.

TABLE V.

Inheritance of habit.

Parents	F_1 phenotypes	F_2 phenotypes	
$s_1s_1S_2S_2$ $S_1S_1s_2s_2$		Spreading	Bunch
Spanish–10 (bunch) × H. G. 1 (bunch) ..	Spreading	235	173
Expected on 9 : 7 ratio		230	178
$\frac{\text{Dev.}}{\text{S. E.}} = 0{\cdot}15$			

The data show that two dominant factors are necessary to produce the spreading type of habit. The genetic constitution of H.G.1 may be taken as $S_1S_1s_2s_2$ and that of Spanish-10 as $s_1s_1S_2S_2$. S_1s_2, s_1S_2 and s_1s_2 phenotypes are bunch in habit and S_1S_2 phenotype is spreading in habit. The constitution of the spreading variety may be taken as $S_1S_1S_2S_2$. This explains 3 : 1 ratio obtained in the crosses between the bunch and the spreading varieties.

The findings are in agreement with the observation made by Badami (1928) that erect is recessive to spreading with the bifactoral difference. Hayes (1933) has, however, reported that the segregation for habit is on the basis of 15 : 1 ratio. He found considerable difficulty in grouping the intermediate plants and, in addition, there were seventeen plants which were abnormal out of the total of 141 plants which he studied. It may be mentioned here that the classification of F_2 population has to be done between six to eight weeks after sowing, otherwise some of the bunch habit plants may, later on, be mistaken for spreading plants. When the bunch plants are sown on a ridge they may, with age, appear spreading in habit. It is, also, essential to sow the seeds at a uniform depth in order to avoid trouble in grouping.

4. *Branching.*—On the basis of branching, the groundnut varieties can be classified into branching and non-branching types. The branching type has two pairs of opposite primary branches developing in the axils of the cotyledons. Each of them has a number of secondary and tertiary branches. Flowers are produced on all these branches. Two or three single primary branches may also appear higher up on the main axis. The non-branching varieties, on the other hand, have only two pairs of primary

branches. In the non-branching type secondary or tertiary branches are not met with, but occasionally a small branch at the junction of the main axis and the primary may appear. Most of the spreading varieties are branched and most of the bunch varieties are not branched. The F_1 of the crosses between the branched and non-branched types were very profusely branched. In F_2 a ratio of three branched to one unbranched was obtained. The data are tabulated below :—

TABLE VI.

Inheritance of branching.

Cross number	Parents	F_1 phenotypes	F_2 phenotypes	
			Branched	Non-branched
25	Branched (BB) Philippine white × Non-branched (bb) Corientes–3	Branched	1050	352
	Expected		1051	351

It is obvious that there is a single factor difference between branching and non-branching. The genetic constitution of the branched and non-branched varieties should be BB and bb respectively. This character has not received the attention of the previous workers.

Linkage between branching and habit.

Since the parents in cross 25, were spreading branched type and bunch non-branched type, the F_2s were tabulated into four groups, *viz.*, spreading-branched, spreading-non-branched, bunch-branched, and bunch-non-branched. The data are tabulated in Table VII.

TABLE VII.

Linkage between branching and habit (30·8 *per cent. crossing over*).

Cross number	F_2 phenotypes			
	Spreading-branched	Spreading-non-branched	Bunch-branched	Bunch-non-branched
25	876	192	174	160
Expected on 9 : 3 : 3 : 1 ratio	788	263	263	88

The figures indicate the absence of 9 : 3 : 3 : 1 ratio. The frequency of the parental combination is more than the expected on the basis of random assortment of gametes : about 30 per cent. crossing over is obtained. This shows that the genes for the habit and branching are different but are located on the same chromosome.

5. *Duration.*—When the groundnut plant matures, the flowering stops and the stem and the branches turn yellow and the leaves are shed, but still some of the pods may remain immature. The early varieties mature within 3 to 3½ months after sowing and the late varieties mature in about 4½ to 5 months. In the crosses between the early and the late types, F_1s seem to be intermediate in duration and F_2s segregate into fairly clear groups of early, medium and late duration plants. By the time when the early plants wither, the medium duration plants have pale yellowish leaves and yellow stems, and the late plants are green. The plants were classified when they were 3½ months old and the classification was not difficult. The actual counts obtained in the F_2 are tabulated below.

TABLE VIII.

Inheritance of duration.

Parents			F_2 phenotypes		
Late LL		Early ll	Early	Medium	Late
Philippine white	×	Corientes–3 ..	313	734	358
Reciprocal ..	..	..	54	119	47
Philippine white	×	Small Japan ..	95	247	114
Philippine white	×	Gudiyattam (bunch) ..	242	538	274
			704	1638	793
Expected on 1 : 2 : 1 ratio ..			784	1567	784

Badami's (1928) finding that earliness is recessive will be true if medium and late plants are grouped together. It is apparent, however, that there is a single factor difference between early (ll) and late (LL).

6. *Pubescence.*—In the groundnut, the stem is generally covered with hairs, the degree of hairiness differing with the variety. Among the varieties under study, two groups were met with. In the first group which is termed hairy, the stem is rather thickly covered throughout with hairs. In

the second type—sparsely hairy—the distribution of hairs is sparse and it is largely confined to the young portions of the stem. The variety, Philippine white, is a typical example of the sparsely hairy types and Corientes-3 is a typical hairy type. In a cross between these two varieties F_1 plants resulted which showed more hairiness than Corientes-3 and in F_2 different gradations of hairiness were met with, but there was no instance of the complete absence of hairiness. The F_2 classification was made on the basis of very hairy, hairy, and sparsely hairy and the figures tabulated in Table IX fit 1 : 2 : 1 ratio. The hairy parent would, therefore, be HH and the sparsely hairy hh. Badami (1928) has also reported that hairiness is dominant over sparsely hairy condition.

TABLE IX.

Inheritance of hairiness.

Parents	F_2 segregation		
Sparsely hairy hh × Hairy HH	Very hairy	Sparsely hairy	Slightly hairy
Philippine white × Corientes-3	300	771	324
Expected on 1 : 2 : 1 ratio..	349	697	349

7. *Seed-coat colours.*—Until very lately only two testa or seed-coat colours, *viz.*, brown or rose, and red, were known to the botanists. The authors were able to obtain in 1932, a sample of kernels having purple coloured testa from Brazil, the home of the groundnut, and a sample from Gambia having white or yellowish grey coloured testa. The last named white variety is supposed to have originated as a mutation in Gambia from the imported Philippine red variety and it is accordingly named Philippine white. The black colour of the seed-coat and the various patterns on the seed-coat, common in many members of the *Papilionaceæ* are absent in the groundnut. It is likely that these homologous characters may be found in some of the wild varieties of the groundnut if a search is made. Vavilov (1922) after a comprehensive study of a large number of genera has enunciated the law of homologous variation. On the basis of homologous characters, the variations in seed-coat colours of the groundnut can be expected.

There are four distinct testa colours in the groundnut, *viz.*, white, rose, red and purple. What is termed here rose is equivalent to the pink or buff or tan or brown of other workers. In Plate IV testa colours obtained in five varieties are shown. For calculating the ratios only four main colours, *viz.*,

rose, red, purple and white were taken into consideration. Different shades of rose colour were grouped under rose, and other colour groups were treated similarly. The following are the seed-coat colours of different varieties used in the crosses mentioned below.—

Variety.	*Testa colour.*
Corientes-3	Dark purple
Small Japan	Red
Saloum	Rose
Gudiyattam bunch	Rose
Philippine white	White

With a view to study the inheritance of testa colours all the possible crosses among the above were made.

Rose colour.—In the crosses 27 and 28 (*vide* Table X) two rose-coloured varieties were separately crossed with Philippine white, the F_1 plants were rose and in F_2 the plants having rose and white testa were in the ratio of 15 : 1. The difference between the white testa and the rose testa is due to two factors R_1 and R_2, either of which produces rose colour in the testa. The constitution of the two rose varieties is, therefore, $R_1R_1R_2R_2$ and that of the Philippine white $r_1r_1r_2r_2$.

TABLE X.

Inheritance of rose testa.

Cross number	Parents	F_1 phenotype	F_2 phenotypes		
	Female (white) $r_1r_1r_2r_2$ Male (rose) $R_1R_1R_2R_2$		Rose	White	
27	Philippine white × Saloum ..	Rose	314	20	Dev. / S. E.
	Expected on 15 : 1 ratio..		313	21	0·23
28	Philippine white × Gudiyattam (bunch)	Rose	996	58	
	Expected on 15 : 1 ratio..		988	66	1·01

Red colour.—In crosses 32 and 33 (*vide* Table XI), rose-coloured varieties were crossed with red-coloured variety—Small Japan. In F_1, red testa was dominant and in F_2 the segregation occurred in 3 : 1 ratio. This is in agreement with the finding of Stock (1910), Badami (1928), Stokes and Hull (1930), and Hayes (1933).

TABLE XI.

Inheritance of red testa.

Cross number	Parents	F_1 phenotype	F_2 phenotypes			
	Rd.Rd. Red rd.rd. Rose		Red	Rose	White	
32	Small Japan × Saloum	Red	49	13		
33	Small Japan × Gudiyattam (bunch)	Red	224	79		
	Observed		273	92		
	Expected on 3 : 1 ratio ..		274	91		
	Expected on 13 : 3 ratio ..		297	68		
	x^2/m		1·939	8·47		P below 0·01
26	rd.rd.$r_1r_1r_2r_2$ × Rd.Rd.$R_1R_1R_2R_2$					
	Philippine white (white) × Small Japan (red)	Red	298	91	19	
	Expected on 45 : 15 : 4 ratio		287	96	25	
	x^2/m		0·420	0·261	1·44	P between 0·5 & 0·3
	Expected on 12 : 3 : 1 ratio		306	77	25	
	x^2/m		0·209	2·555	1·440	P between 0·2 & 0·1

Crosses 32 and 33 indicate that there is a single factor difference between red and rose. In cross 26 (Small Japan × Philippine white) we find that rose-coloured kernels appear in F_2, indicating that Small Japan carries the factors for both the rose and the red colours. The number of factors for rose, present in Small Japan, is a point for consideration. The F_2 segregation of the progeny of cross 26 is either according to 45 : 15 : 4 ratio or 12 : 3 : 1 ratio. There are two hypotheses possible, *viz.*, (i) that the red colour in the testa develops only in the presence of one of the rose factors, and (ii) that the expression of the red factor is independent of the presence of a rose factor.

Taking the first hypothesis, there are two possibilities according to the number of rose factors present in Small Japan.

	Small Japan		Philippine White	F_1	F_2 Red : Rose : White
(*a*)	Rd.Rd.$R_1R_1R_2R_2$	×	rd.rd.$r_1r_1r_2r_2$	Rd.rd.$R_1r_1R_2r_2$	45 : 15 : 4
(*b*)	Rd.Rd.$R_1R_1r_2r_2$	×	rd.rd.$r_1r_1r_2r_2$	Rd.rd.$R_1r_1r_2r_2$	9 : 3 : 4
	or				
	Rd.Rd.$r_1r_1R_2R_2$	×	do.	Rd.rd.$r_1r_1R_2r_2$	

Since 9 : 3 : 4 ratio is not obtained, the proposition under (*b*) is ruled out.

Under the second hypothesis, there are the following two possibilities :—

	Small Japan		Philippine White	F_1	F_2 Red : Rose : White
(*c*)	Rd.Rd.$R_1R_1R_2R_2$	×	rd.rd.$r_1r_1r_2r_2$	Rd.rd.$R_1r_1R_2r_2$	48 : 15 : 1
(*d*)	Rd.Rd.$R_1R_1r_2r_2$	×	do.	Rd.rd.$R_1r_1r_2r_2$	12 : 3 : 1
	or				
	Rd.Rd.$r_1r_1R_2R_2$	×	do.	Rd.rd.$r_1r_1R_2r_2$	

The proposition under (*c*) is ruled out since the ratio obtained is far from 48 : 15 : 1. The choice is between the propositions (*a*) and (*d*). If the correct ratio is 12 : 3 : 1, the assumption should be that Small Japan carries one rose factor but its presence is not necessary for the development of the red colour. On the other hand, if the ratio 45 : 15 : 4 is the correct one it will have to be assumed that the rose factor is necessary for the development of red and that Small Japan carries both the rose factors. From the analogy of the results obtained for the purple testa and from the inheritance behaviour of the pigment in the vegetative portions of the plant, the latter assumption appears to be the more plausible one.

Purple colour.—In crosses 30 and 31 (*vide* Table XII) the plants having purple testa were crossed with those having rose testa. F_1 was purple and in F_2 the segregation was in the ratio of 3 purples and 1 rose. This indicates a single factor differences between the rose-coloured varieties and the purple, and that Corientes–3, the variety having purple testa, carries factors for rose also.

That purple is dominant over red and that there is a bifactorial difference between the red and the purple is evident from cross 29, where segregation in F_2 occurs in the ratio of 12 purples, 3 reds and 1 rose. PRd.R and Prd.R phenotypes are purple, PRd.R are red, and p.rd.R are rose. The cross 25 shows the absence of the factor Rd. in Corientes–3.

The interpretation of the F_2 results of cross 25 will depend upon what we assume about the relationship between the purple and the rose factors. If it is assumed that purple carries one of the rose factors and that purple

TABLE XII.

Inheritance of purple testa.

Cross number	Parents	F_1 phenotype	F_2 phenotypes			
	PP × pp Purple Rose		Purple	Rose		
30	Corientes–3 × Saloum ..	Purple	195	81		
31	Corientes–3 × Gudiyattam (bunch)	Purple	71	21		
	Total observed		266	102		
	Expected on 3 : 1 ratio ..		276	92		
	x^2/m		0·362	1·086		P between 0·2 & 0·3
	Expected on 13 : 3 ratio ..		299	69		
	x^2/m		3·642	15·79		P. below 0·01
	PPrd.rd. × pprd.rd Purple Red		Purple	Red	Rose	
29	Corientes–3 × Small Japan ..	Purple	280	66	25	
	Expected on 12 : 3 : 1 ratio..		278	70	23	
	x^2/m		0·014	0·229	0·174	P between 0·8 to 0·9
	Expected on 45 : 15 : 4 ratio		261	87	23	
	x^2/m		1·384	5·069	0·174	P between 0·05 & 0·02
	PPrd. rd. $R_1R_1R_2R_2$ (Purple) × pprd. rd. $r_1r_1r_2r_2$ (white)		Purple	Rose	White	
25	Corientes–3 × Philippine white	Purple	1056	364	99	
	Expected on 45 : 15 : 4 ratio		1061	354	94	
	x^2/m		0·024	0·282	0·266	P between 0·8 & 0·7
	Expected on 12 : 3 : 1 ratio		1140	285	94	
	x^2/m		6·190	2·190	0·266	P between 0·3 & 0·2

can show only in the presence of the latter factor, the F_2 ratio should correspond to 9 : 3 : 4 of purple : rose : white, but the actual ratio obtained obviously does not represent this. The F_2 ratio can be taken as either 12 : 3 : 1 or 45 : 15 : 4 of purple : rose : white. If we accept the former ratio as the correct one, we should assume that Corientes-3 carries one rose factor but its presence is not necessary for the expression of purple factor. On the other hand, if the ratio 45 : 15 : 4 is taken as correct, it is necessary to assume that the rose factor is necessary for the development of purple and that Corientes-3 carries both the rose factors. The latter assumption appears to be the more plausible one.

8. *Anthocyanin pigment.*—The presence of purple pigment on the stem in varying intensity is a common occurrence in the groundnut varieties. Even though the stem may show slight traces of the pigment, it may be noticed very much more intensified on the tender developing gynophores. Among 100 varieties collected so far, there is only one variety, namely, Philippine white with kernels having white testa and which shows complete absence of purple pigment and the plants are green throughout. In crosses involving a purple pigmented parent and a green parent, the F_1 is purple pigmented. In F_2 the segregaation occurs into purples and greens in 15 : 1 ratio (*vide* Table XIII). Among the purples, different degrees of pigmentation

TABLE XIII.

Inheritance of anthocyanin pigment.

Cross number	Parents	F_1 phenotype	F_2 phenotypes	
	$r_1r_1r_2r_2$ × $R_1R_1R_2R_2$		Purple	Green
	Green × Purple			
25	Philippine white × Corientes-3 ..	Purple	1295	98
26 (*a*)	Do. × Small Japan ..	,,	432	24
27	Do. × Saloum ..	,,	315	21
28	Do. × Gudiyattam (bunch) ..	,,	996	58
	Observed		3038	201
	Expected on 15 : 1 ratio		3037	202
			F_3 segregation	
				Dev.
	F_2 Genotypes of cross 26 (*a*)		Purple Green	S. E.
	$R_1r_1r_2r_2$ and $r_1r_1R_2r_2$ ..		201 52	
	Expected on 3 : 1 ratio		190 63	1·59
	$R_1r_1R_2r_2$ Dihybrids ..		183 9	
	Expected on 15 : 1 ratio		180 12	0·88

are observed. Hayes has reported a single factor difference between purple and slight purple.

Anthocyanin pigment and seed-coat colours.—The plants which were studied for testa colours were also observed for the purple pigment in the plant. It was found that only plants having white testa showed the absence of purple pigment on the gynophores and branches. The plants having purple pigment had invariably coloured or pigmented testa. Even in the F_3 plants with purple pigment on the vegetative parts white or colourless testa could not be found. All plants lacking in anthocyanin pigment had invariably white coloured seed-coats.

The interpretation of the results would depend upon what we assume about the relationship between the factors for seed-coat colours and the anthocyanin pigment in the plant. In the absence of even a few crossovers among the large population studied, it cannot be readily postulated that the factors for plant colour are linked with the seed-coat colour factors, and that too with all the four factors P, Rd, R_1 and R_2. Even though the assumption that the two factors for plant colour are absolutely linked with two seed-coat colour factors R_1 and R_2 is adequate to explain the results, it appears unnecessary in view of the observations recorded in other species. Emerson (1921) has found in maize that the factors A, C and R affect both the plant colour and the aleurone colour. The factor A develops anthocyanin pigment in the plant and in the aleurone also. On analogy with the relationship between plant colour and aleurone colour factors in maize, it might be assumed that the factors R_1 and R_2 produce purple pigment in the plant and rose colour in the seed-coat. Since the plant colour and the seed-coat colours are doubtless anthocyanin pigments, it seems to be natural to expect close inter-relationship between them. Consistently with this hypothesis it must be assumed that the varieties with coloured seed-coats which were investigated, carry both the rose factors and that only in the presence of a rose factor P or Rd. is expressed. On this basis the genetic constitution of the varieties studied is summed up below :—

Colour of seed-coat	Variety	Genotype
White	Philippine white	pprd.rd.$r_1r_1r_2r_2$
Rose	Gudiyattam bunch	pprd.rd.$R_1R_1R_2R_2$
Rose	Saloum	Do.
Red	Small Japan	ppRd.Rd.$R_1R_1R_2R_2$
Purple	Corientes-3	PPrd.rd.$R_1R_1R_2R_2$

Discussion.

Waldron (1919) has suggested that *Arachis hypogæa* may be divided into two sub-species, *viz.*, *fastigiata* for the bunch type and *procumbens* for the spreading type. He postulates that " the cultivated bunch varieties are derived from such species as *A. pusilla*" a wild Brazilian species, erect in habit, and that " the prostrate varieties are derived from *A. prostrata*," another wild species in Brazil which is spreading in habit. The varieties Gudiyattam bunch, Small Japan, and Spanish-10 would come under the sub-species *fastigiata* ; and the spreading varieties Philippine white, Saloum and Madagascar would come under the sub-species *procumbens*. In tracing the phylogeny of the sub-species *fastigiata* and *procumbens*, a comparison of the genetic complex of each of these two sub-species would be valuable. One of the objects of the geneticist's enquiry is to discover, if possible, what the distinctions between species are in terms of genetic constitution.

TABLE XIV.

Genetic analysis of the sub-species procumbens *and* fastigiata.

Characters	Gene complex		Characters
	Procumbens	*Fastigiata*	
Spreading habit ..	$S_1S_1S_2S_2$	$s_1s_1S_2S_2$	Erect or bunch habit
Branching habit ..	BB	bb	Non-branching habit
Chlorophyll deficiency factors	$G_1G_1g_2g_2$	$g_1g_1G_2G_2$	Chlorophyll deficiency factors
Long duration	LL	ll	Short duration
Abnormality factors ..	$N_1N_1n_2n_2$	$N_1N_1N_2N_2$ $n_1n_1N_2N_2$	Abnormality factors Do. in some varieties
Basic testa colour—factors for rose and for anthocyanin pigment in plant	R_1R_1	R_1R_1	
Do. in one variety..	r_1r_1	..	
Red testa factors ..	Rd. rd.	Rd. rd.	
Purple testa factors ..	p	p p	

The tabulation of the genic analysis of the two sub-species shows that the differences between them are not wide. Similar differences are found among the varieties in many species, and generally no attempts are made to group such varieties into sub-species or to suggest their origin from two different species. The so-called sub-species *fastigiata* and *procumbens* cross very readily, and the number of chromosomes in both the sub-species is the same. Until it is proved that the behaviour of the chromosomes of these two sub-species is different, the necessity for postulating separate origin for these two species is doubted.

A reference to the literature dealing with the studies regarding the inheritance of characters in the different members of *Leguminosæ* shows that in most of the species studied branching forms are dominant over non-branching forms and that erect or bunch habit is recessive to the spreading. The parallel behaviour of these characters is suggestive of true homology.

Summary.

In an attempt at a genetic analysis of the several characters—chlorophyll deficiency, abnormality, habit, branching, duration, hairiness, anthocyanin pigment in the plant and four seed-coat colours—of the groundnut, data accumulated during a period of five years, involving an examination of not less than 10,000 individual plants are reported. As an interpretation of the results obtained, thirteen genetic factors are assumed and the following conclusions are drawn :—

1. When a bunch ($g_1g_1G_2G_2$) variety is crossed with a spreading ($G_1G_1g_2g_2$) variety the segregation occurs in F_2 in the ratio of 15 green to one albino plants.

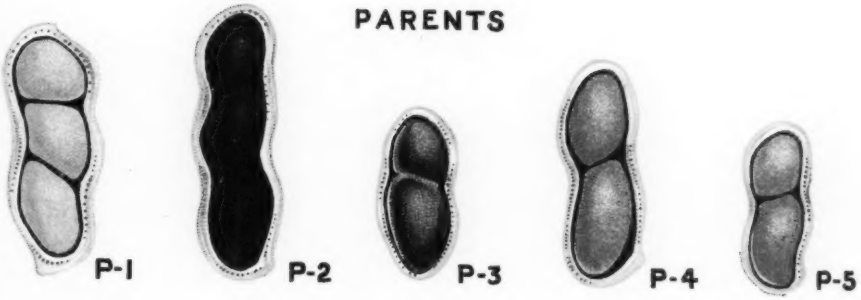
2. Abnormal dwarf plants which are mostly sterile, were obtained in a cross between the spreading varieties and the bunch variety Corientes-3. There are two factors for abnormality which give 15 : 1 ratio in F_2.

3. The spreading habit is met with when the dominant factors (S_1S_2) are present. In the absence of either one or both of them the progeny is bunch in habit.

4. The character—branching is dominant over non-branching and is inherited in simple Mendelian ratio of 3 : 1 in F_2. The branching is linked with the spreading habit with 30 per cent. of crossover.

5. Late duration is dominant over early. The F_1 is intermediate in duration and F_2 segregation is in the ratio of 3 non-early and one early plants.

6. Hairiness is dominant over sparse hairiness and is caused by a single gene difference.


PARENTS
P-1
P-2
P-3
P-4
P-5

7. There are four distinct seed-coat colours, in groundnut, *viz.*, dark purple, red, rose and white. There are two duplicate factors for the rose colour. The factor for red and the factor for purple are dominant to rose. But they (red and purple factors) are expressed only in the presence of the rose factor. The purple is dominant to red. The white seed coat colour (pprd.rd.$r_1r_1r_2r_2$) is recessive to the coloured seed-coats.

8. The purple pigment in the plant is produced by duplicate genes which give in F_2 15 : 1 ratio. It is suggested that the two factors which produce the rose coloured testa might also produce the purple pigment in the plant.

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EXPLANATION OF PLATE IV.

P-1 : Philippine white ; P-2 : Corientes-3 ;
P-3 : Small Japan ; P-4 : Saloum ; P-5 : Gudiyattam bunch.

A NEW SPECIES OF THE NEMATODE GENUS *DERMATOXYS* FROM *LEPUS RUFICAUDATUS*.

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The worms belonging to the genus *Dermatoxys* Schneider, 1866, are said to have a wide distribution but these have seldom been noted. *Dermatoxys veligera* was described from Brazil in 1866 and not again noted for almost half a century when Seurat (1915) discovered its existence in Algeria and also described *D. getula* from the cæcum of *Xerus getulus* in Mogador, Morocco, and Hall (1916) obtained *D. veligera* from *Lepus californicus melanotis* in Oklahoma, U. S. A.

Out of the several hares which were sacrificed in the Zoological laboratories, Aligarh, only one was found to be infected with *Dermatoxys species* and two males and thirteen females were recovered from the colon of *Lepus ruficaudatus*.

Morphology.—The worms are white, stout and almost straight. The caudal extremity of the males is moderately curved ventrad. The cuticle shows fine transverse striations. There are two cervical alæ which may extend to a point beyond the œsophageal bulb, terminate at the level of the œsophagus or end a little above it. The mouth possesses three well-developed lips, each bearing two papillæ. The vestibule is short and is provided with three teeth. The œsophagus, exclusive of the bulb, is widened anteriorly to form a short thick portion and diminished posteriorly to form a long portion which terminates in a bulb.

Males.—11·5 × 0·44 mm. Both the males are of equal length and thickness. The œsophagus, inclusive of the bulb, is 1·23 mm. long and the cervical alæ in one male specimen are 1·54 mm. in length (Fig. 1) and in the other extend to a little above the œsophageal bulb. The tail ends in a blunt point with a cuticular thickening at its tip on the ventral side. This is very distinctly seen in a lateral view of the caudal extremity (Fig. 2). The caudal bursa is formed of two very long and wide alæ, 1·21 mm. in length. These are continued round the posterior end of the tail. On the ventral surface, at a distance of 0·88 mm. anterior to the cloaca, there is

a longitudinal row of transverse comb-like structures about thirteen in number. There are three rows of cuticular bosses converging above the cloaca and running upwards on the ventral side. Along the posterior lip of the cloaca, there is a group of three papillæ of which the median is the unpaired and at the sides of these there is a pair of very large and conspicuous

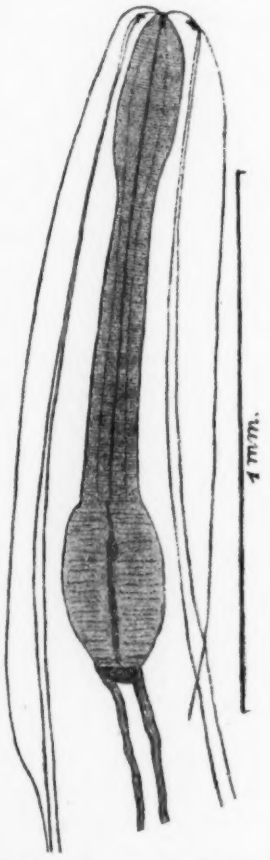

FIG. 1. *Dermatoxys ruficaudata* n. sp. Anterior extremity of male.

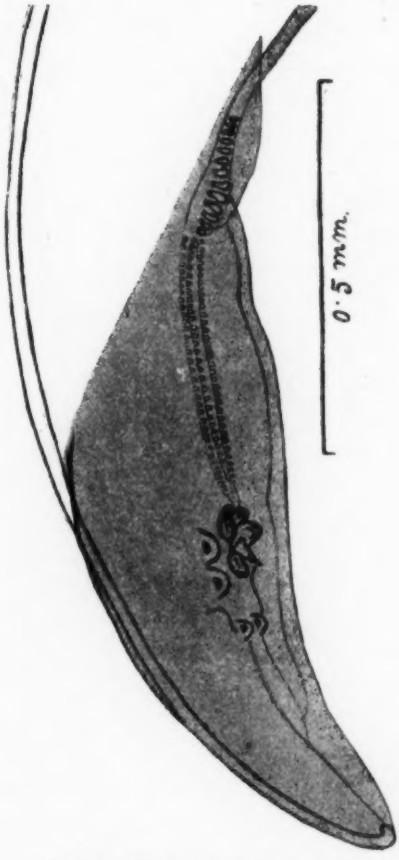

FIG. 2. *Dermatoxys ruficaudata* n. sp. Posterior extremity of male, lateral view.

papillæ. The same arrangement is repeated along the inferior lip of the cloaca. At the beginning of the caudal end there is a single pair of large papillæ. A little above these lies a pair of cuticular shields (Fig. 3). The spicule has not been observed in either of the two males.

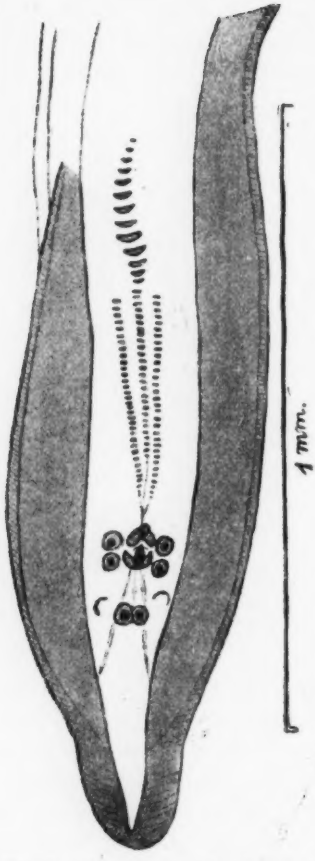

FIG. 3. *Dermatoxys ruficaudata* n. sp. Posterior extremity of male, ventral view.

Females.—16·1 × 0·60 mm. to 17·1 × 0·62 mm. In a female measuring 17·1 mm. the œsophagus is 1·73 mm. long and the cervical alæ are 1·62 mm. in length. The inconspicuous vulva lies at a distance of 7·54 mm. from the anterior end. The two ovaries are massive and deeply coloured. They lie in the anterior part of the body and communicate by narrow S-shaped oviducts with the long receptacula which unite in the vicinity of the anus to form the single uterus. This extends anteriorly to the vagina. The eggs measure 0·11 × 0·06 mm. They possess thick shell and plug at one pole. The tail is long and gradually tapering.

Conclusion. — The genus *Dermatoxys* Schneider, 1866, embraces three species : *D. veligera* (Rudolphi, 1819) Schneider, 1866 ; *D. getula* Seurat, 1915 ; and *D. polyoon* Linstow, 1909. The species discovered by the writer of this paper differs markedly from the two last named species but in certain respects resembles the first named and is to be distinguished from it by the number and arrangement of the ventral caudal papillæ. Although the description of *D. veligera* given by Schneider, Seurat, Hall and the figures given by Yorke and Maplestone all differ from one another in some respect or the other, yet none of the description is applicable to the species found in *Lepus ruficaudatus*. This necessitates the creation of a new species and it is proposed to call it *Dermatoxys ruficaudata*.

It is interesting to note that the cervical alæ in certain specimens extend beyond the œsophageal bulb while in others they are shorter and end above it or terminate at the level of the œsophageal bulb. Hall has observed the first condition and repudiates Seurat's statement that the cervical alæ in *D. veligera* terminate posteriorly at the level of the œsophageal bulb. Yorke and Maplestone have mentioned that the cervical alæ extend from the head to the end of the œsophagus but from the figure given by them it appears that

these end above the œsophageal bulb. The writer has observed all the three conditions in the material obtained by him and concludes that the length of the cervical alæ may differ in males and females, both of the same species.

The specimens have been deposited in the Museum of the Zoological Laboratories, Muslim University, Aligarh, U. P., India, No. 939. Type Slides Nos. 940–944.

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PLANT GROWTH IN RELATION TO PARTIAL PRESSURES OF OXYGEN.

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AND
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(From the Institute of Agricultural Research, Benares Hindu University, Benares.)

Received October 21, 1935.

Introduction.

For some time past, work has been in progress at this Experiment Station to analyse the influence of environic complex upon plant growth in these sub-tropical regions where so little is known as to the special adaptations which some plants seem to possess to cope with the atmospheric conditions to which they are exposed. In conducting such studies, it is observed that of the atmospheric variables—besides temperature, solar radiation, and humidity—the concentration of oxygen and carbon-dioxide seems to greatly influence the growth of plants. The importance of these two constituents of air is made evident from the fact that whereas carbon-dioxide serves as a means for the manufacture of those organic materials which ultimately go to build up the entire plant body, oxygen functions as an energy liberator by its oxidising action on the labile and easily oxidisable components of the protoplasm and thus supplies the necessary energy for all the vital activities of the plant. While the influence of CO_2 on plant growth has been studied by a number of workers specially Cummings and Jones,[2] Kuiper,[5] Owen and Williams[6] besides others investigating on the CO_2 fertilisation, our knowledge in regard to the relationship between oxygen and the growth of plants seems to be too meagre to provide any definite evidence as to the response made by the growth of plants to varying concentrations of oxygen.

The present work aims at investigating the relationship which oxygen holds to the growth of plants when raised under controlled experimental conditions. The plants under investigation are raised under as natural yet standard conditions as possible, such that except varying the individual factor—the oxygen concentration—the influence of which is under investigation, the rest of the factors are supplied well above their limiting value. The potted plants are allowed to grow under glass chambers at a constant

temperature, and their growth at successive intervals estimated in terms of dry matter produced.

Experimentation.

Wheat seeds (*var.* Pusa 4) of the same average density and size are sown in large sized pots fully supplied with manurial constituents accompanied by the required watering and hoeing. After the plants have attained an age of fourteen days, in the fully lighted and well ventilated experimental laboratory, they are administered varying doses of oxygen and made to grow under constant yet non-limiting conditions of external variables. In order to keep the work as far as possible within the limits of least variation, fully established seedlings, fourteen days old, are selected for investigation and the experiments continued only till such time that preparatory changes leading to the initiation of the reproductive organs do not introduce a fresh variable.

Variation in the concentration of oxygen is accomplished by diluting the gas with nitrogen. For obtaining a known concentration, the two gases, after passing through a battery of purifiers are mixed in large-sized containers in the required proportion by the usual method of the displacement of water. The gases so mixed in the container pass out by means of an exit tube into a carbon-dioxide generating tower where they are mixed with the requisite quantity of carbon-dioxide generated by the action of hydrochloric acid on marble. The oxygen-nitrogen mixture thus contaminated with carbon-dioxide in the tower, passes first through a catch bottle containing some water and later through the plant chambers. The gas mixture is analysed from time to time with regard to the percentage of O_2 and CO_2 by withdrawing a sample of the gas from the catch bottle into the burette of the Haldane Air Analysis apparatus. The percentages of oxygen and carbon-dioxide are thus experimentally determined at regular intervals and arrangements made to maintain them under as standard conditions as possible. In all, five concentrations of oxygen namely, 6, 11, 15, 19 and 21 per cent. are used, no necessity being felt to work below or above these concentrations as independent observations showed that by so doing complications are introduced in other directions specially in the mechanism of respiration.[8] The concentration of CO_2 is maintained at 0·35% which concentration has been found by independent observations[9] to be optimum for assimilation of wheat leaves.

The plant chambers consist of square boxes having polished crystal white glass on all sides, except at the bottom where they are fixed to a thick glass sheet. Inlet and exit tubes are provided on the sides while a thermometer and a manometer are fixed on the upper side. Uniform illumination

is maintained by an overhead Osram lamp of a thousand watts kept at a distance which did not allow of rise in temperature inside the chambers. That this light fully depleted the CO_2 concentration at which the plants grew is previously determined as detailed in an independent investigation on the relation of light to photosynthesis.[9] The temperature is maintained constant at 31°C. For each concentration of O_2 used the plants are raised in separate chambers so that without disturbing the concentration inside the chambers under a given group, the plants can be taken out in succession every third day for the appreciation of dry weight.

In brief, the general procedure of work consists in taking the entire population of plants grown under a particular chamber, washing them free from superficial contamination, and finally determining the dry weights after repeated drying at 100°C. Particular care is taken to include a large number of plants for each oxygen treatment so that the mean value per plant in each of the several lots taken from any one group showed differences statistically significant.

Experimental Findings.

On examining the relation between oxygen supply on the one hand, and dry matter production on the other, with respect to age it becomes evident from Fig. 1 that in the first instance, the production of dry matter goes on continuously increasing with increasing oxygen concentration, irrespective of the age of the plants, emphasising thereby that the growth is essentially limited by the oxygen supply and that increase in dry matter production is more or less directly correlated to the oxygen concentration till a certain range.

While concentration of oxygen is found to influence the dry matter production, growth is marked to be greatly altered with the age of the plants when taken in conjunction with the concentration of oxygen. Curves No. IV and V (Fig. 2) depicting the behaviour of plants exposed to 6 and 11% are characteristic and indicate a slow increase in dry matter production, attaining the highest observed level when the plants are twenty-three days old, and with the further advance in age show a decline. In conformity to expectations, the amount of matter added is greater under 11% than under 6% as discussed above.

This decrease in dry matter production in response to a corresponding decrease in oxygen concentration cannot but be ascribed to variations introduced in the metabolic activities of the plants. The decline in absolute dry matter may probably be brought about by a lowering of the photosynthetic activity under low concentrations of oxygen or may be due to decreased respiration against lesser percentage of oxygen as was observed

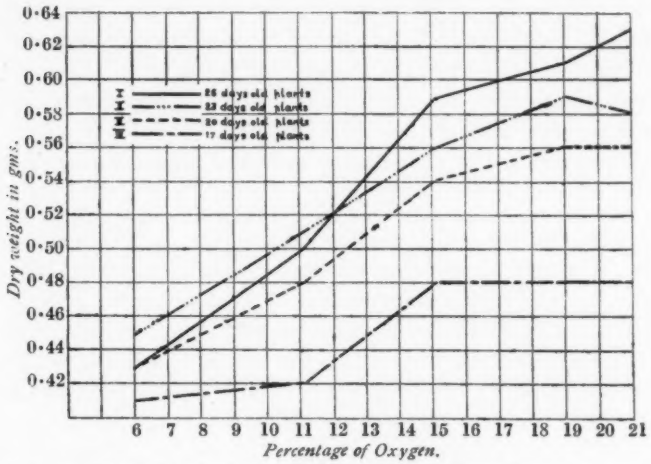

FIG. 1.—Dry weight of wheat plants under varying percentages of oxygen.

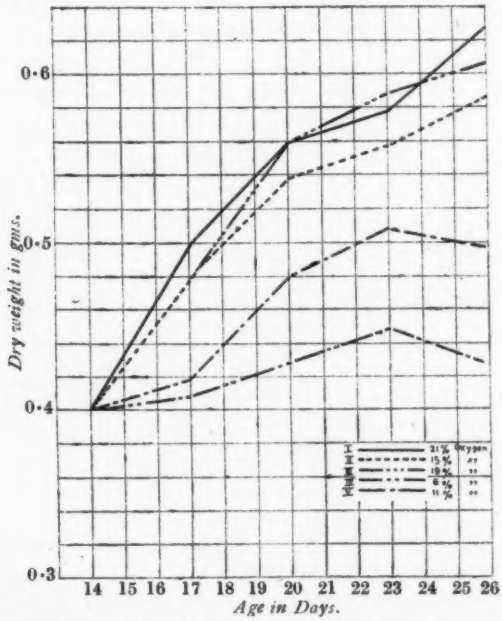

FIG. 2.—Dry weight of wheat plants at successive stages of growth during the juvenile phase of life cycle.

by Boussingault[1] and Pringsheim.[7] Observations of Ewart[4] on mosses indicate that photosynthesis declines as the oxygen concentration is decreased while

Willstätter and Stoll[12] on the other hand maintain that the partial pressure of oxygen can be reduced to 1/100th of that in the air without disturbing photosynthesis, if the rest of the atmosphere is nitrogen. Complete displacement of oxygen, however, checked the activity altogether even when all other factors were above the limiting value. Warburg,[11] on the contrary, records a decrease in the assimilatory rate with increasing concentrations of oxygen.

As to the relation which respiration holds to variations in oxygen concentration, it is well known that anærobiosis, though partial, is introduced by a decrease in oxygen concentration. The decrease in growth, therefore, may be ascribed to a decrease in the available energy under such conditions of partial anærobiosis leading to poor growth and early death of plants. It is also possible that the early death of the plants is brought about by an accumulation of harmful metabolites and production of certain toxic substances during anærobiosis as has been observed in a separate communication.[8]

In explaining the curves on the basis of these two metabolic activities the fact has to be borne in mind that as the experimental plants belong to the juvenile stage of development, the rate of assimilation is expected to be low even under normal oxygen concentrations as revealed in a parallel study on these very leaves,[10] and hence a lowering in the assimilatory rate under sub-normal percentages of oxygen cannot by itself adequately explain the observed variation in growth. It may thus be possible that the development of photosynthetic machinery during the early stages is relatively slow under such conditions of low oxygen concentrations as compared to that at higher ones, leading to a low photosynthetic efficiency and a low rate of construction of materials and hence decreased growth. The influence on growth under such conditions may, therefore, be only of an indirect nature. On the contrary, in a parallel independent study[8] it is inferred that lack of oxygen initiates a slackening in the respiratory activity, which being thus reduced, the early growth of plants is checked leading to poor development of roots, stem, leaves and other tissues, and consequently poor growth.

Thus with the increase in the concentration of oxygen there follows a proportionate increase in dry matter production. In case of plants of younger age (Curve No. IV, Fig. 1) the proportionate increase is characteristic upto 15% after which the curve merges into a more or less horizontal phase indicating thereby that during earlier stages of growth optimum values for the wheat plants lie near about 15%, further increase beyond which does not seem to increase the addition of dry matter proportionately. At later

stages (Curves No.I-III, Fig. 1) the level phase, however, is not so characteristic. The production of dry matter with advance in age becomes more or less proportional to the oxygen concentration, the change in behaviour being brought about probably by the age-accruing variations in the internal complex of the plant machinery.

These facts lead one to conclude that small variations in the atmospheric concentrations of oxygen upto a minimum of 15% in nature do not materially affect the growth of plants to any great extent.

On re-examining the two figures referred to above it becomes obvious that the increase in the dry matter production whether in relation to age or oxygen concentration provides curves which are characteristically similar to those of optima and limiting factor curves obtained in relation to CO_2 concentration, temperature, and light intensity in case of photosynthesis for these very plants.[9] The growth thus varies, on the one hand, with age of the plant, and on the other, with the concentration of oxygen used. There are obviously, therefore, two factors at work in the present case, one dealing with the age—the age-factor, while the other with the oxygen concentration, the dry matter produced being the resultant of the interaction between the two.

Figs. 1-2 dealing with the age-factor in consonance with the factor for oxygen shows that the dry matter produced at any time differs under different concentrations. The higher the concentration, the greater is the production of dry matter, probably on account of greater oxygen supply resulting in the release of greater amount of energy for the growth of plants. Under 6 and 11% the curves go on ascending for some time and later show a decided fall, whereas under 15, 19 and 21% only the ascending phase is explicit. Till the 23rd day growth is the function of both these factors, namely, the oxygen supply and the age-factor but at this stage probably the age-factor becomes above the limiting value, the rate being governed by the oxygen concentration. Under higher concentration, however, the age-factor is still limiting as shown by an increase in dry matter produced with the age of the plant.

At any point on the curve the growth of the plants can be increased by an increase in either the age-factor or the oxygen concentration. Growth at any stage is limited by not one factor alone but at least two. How and in what way do the two factors dealing with the age of the plant and the oxygen concentration operate in producing changes in the absolute dry matter production in different sets, cannot be said with certainty at present. But the observations recorded here as well as outside go to show that both the assimilatory and respiratory activities change with the age of the plant as also

the intensity of oxygen supply, emphasising thereby that the differences may, to a large extent, be accounted for on the basis of these two metabolic activities.

Conclusions.

The paper deals with the effect of varying concentrations of oxygen on the production of dry matter in plants. The following conclusions are the outcome of the present investigation :—

The dry matter produced is more or less proportional to the concentration of oxygen. With increasing percentages the curve merges into a stationary and later a decline phase.

The increased growth at higher concentrations of oxygen is explained on the basis of increase in the intensity of both respiration and assimilation. Suggestion is made that the former controls the production of dry materials probably by a control over the supply of energy, while the latter by controlling the development of the photosynthetic machinery.

The deviations in the concentration of Oxygen near about those of the atmosphere do not affect the dry matter production to any great extent, proving thereby that growth is not ordinarily limited by the concentration of oxygen under natural conditions of the plains and the critical concentration for the growth of wheat plants lies near about 15%.

The dry matter production at any stage appears to be a function of the interaction between two variables, the age-factor and the factor for oxygen. An increase in any one of these factors at any stage brings about increased growth showing thereby that under the conditions of experimentation growth in plants at any stage of the life-cycle is not limited by one factor alone but by as many as two factors, *viz.*, the age-factor and the factor for oxygen.

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CONTRIBUTION TO THE LIFE-HISTORY OF *CEDRUS DEODARA* LOUD.

I. The Development of the Pollen Grains.

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Received December 11, 1935.
(Communicated by Dr. P. Maheshwari, D.Sc.)

1. Introduction.

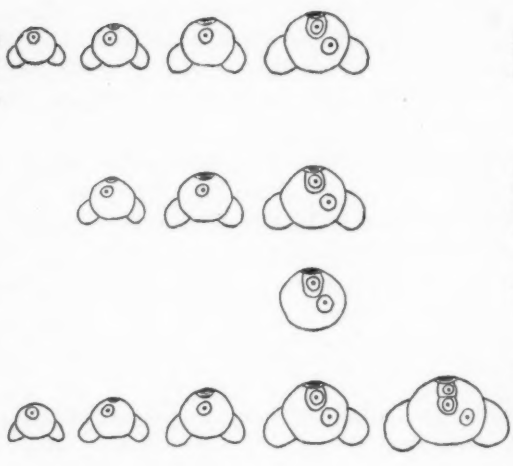
In the year 1930, Dr. P. Maheshwari began collecting some material of *Cedrus deodara* with the object of studying the life-history of this interesting Indian Conifer. Due to the difficulties of continuing the collections in the various seasons, this work was held up for a long time and even now only the development of the microspores and the pollen grains can be described. Many important stages in the formation of archegonia and embryos have also been obtained, but some are still missing and consequently an account of this part has to be postponed for the future.

The material used for this investigation was collected from Mussoorie and Simla.[1] Formalin-acetic alcohol was the only fixative that could be used. The young cones were pared on opposite sides and the larger ones were either cut into smaller pieces or dismembered ; in the case of the oldest cones the pollen was shaken out and dropped into the killing fluid. The usual methods of dehydration and imbedding were followed and sections were cut 5-15 microns thick. Some imbedded material and prepared slides were very kindly handed over to me by Dr. P. Maheshwari. Haidenhain's Iron-alum hæmatoxylin, with Fast Green as a counter stain, gave the best results. Although the fixation was quite adequate for morphological study, the finer details of reduction divisions could not be made out due to a clumping of the chromosomes and shrinkage of the cytoplasm.

2. Historical.

A large amount of work has already been done on the development of the male gametophyte of the *Coniferales*, so that most of the genera are now more or less well known. It is not possible to review the whole of this

[1] My thanks are due to Messrs. Raghubir Prasad and H. L. Gulatia, who kindly fixed the material from Simla. A part of the Mussoorie material had been fixed by the late Dr. Winfield Dudgeon and handed over to Dr. P. Maheshwari for investigation.



Genus & Author	Micro-spore	1st division	2nd division	3rd division	4th division	Remarks
1. *Pinus laricio* (Chamberlain, 1901) *P. strobus*, *P. rigida*, *P. austriaca*, *P. montana* var. *uncinata*, *P. resinosa* (Ferguson, 1904) *P. palustris* (Mathews, 1932)						Sethi (1928) reported 3-celled pollen grains in *P. longifolia*, but this has been shown to be incorrect (Johri, 1935).
2. *Keteleria fortunei* (Hutchinson, 1917)						There are many variations. In one case 3 prothallial cells were seen while in another 4 cells were situated in the centre of the pollen grain.
3. *Pseudotsuga douglasii*, (Lawson, 1909)						Only mature pollen grains were seen. A fuller investigation would be desirable.
4. *Picea excelsa* (Miyake, 1903) *P. canadensis* (Hutchinson, 1915)						Miyake observed only the mature stages. According to Hutchinson the number and arrangement of nuclei show frequent variations. Double pollen grains occur.
5. *Pseudolarix Kaempferi*, (Miyake & Yasui, 1911)						
6. *Abies balsamea*, (Hutchinson, 1914)						Frequent variations occur in the number of prothallial cells. In about 10% cases the spermatogenous cell divides to form two male nuclei *before* the pollen is shed.
7. *Cedrus atlantica* (Smith, 1923)						Smith's description is very brief and illustrated with only two figures of the mature pollen grains. Further details of the development of the male gametophyte in *C. deodara* are described in the present paper.
8. *Larix europaea* (Coker, 1904) *L. leptolepis*, (Doyle, 1918)						Only the mature pollen grains have been described. Coker figures only one prothallial cell but evidently he missed the other, for Doyle saw both. Several variations occur, double pollen grains being frequent.
9. *Tsuga* (According to Smith, 1923)						The development has not been investigated in this genus, but the pollen grains are wingless except in *T. pattoniana* and there is a statement in Smith's paper (1923) that they are five-nucleate.

FIG. 1.—A diagrammatic representation of the development of the male gametophyte in the *Abietaceae*.

literature here, but so far as the family *Abietaceæ*[2] is concerned, the nine genera may be divided into two groups :—

1. The first group includes those genera in which the pollen is shed at the four-nucleate stage—two prothallial cells, an antheridial cell and the tube nucleus :—*Pinus*, *Keteleeria* and *Pseudotsuga*.

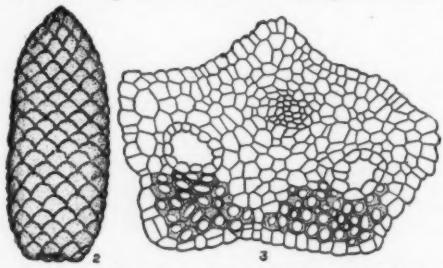
2. The second group includes those genera in which the pollen is shed at the five-nucleate stage—two prothallial cells, one sterile cell[3] (stalk cell), the spermatogenous cell (body cell) and the tube nucleus :—*Picea*, *Pseudolarix*, *Abies*, *Cedrus*, *Larix*, and *Tsuga* (?).

The more important facts in this connection are given in the accompanying chart (Fig. 1).

The earliest work on *Cedrus* is that of Saxton (1921), who published a preliminary note on the life-history of *Cedrus deodara*, giving the approximate dates for flowering and fruiting. Further work could not be continued, as Saxton left India soon after. Smith's (1923) work on *C. atlantica* gives more information, but this deals mainly with the female gametophyte and embryo.

3. Male Cones.

The staminate cones are solitary, terminal and erect. Each is a cylindrical spike, measuring from $1\frac{1}{2}$ to 2 inches in length when mature (Fig. 2)[4].



Figs. 2-3.—Fig. 2, A mature male cone, approximately natural size. Fig. 3, T.S. of a young microsporophyll. The two groups of stippled cells will give rise to the microsporangia. Note the resin canals above each group. × 162.

There are numerous spirally arranged microsporophylls situated so close to one another that the entire cones are very difficult to section (Fig. 6). There are two sporangia on the abaxial side of each microsporophyll and the connective is prolonged into a denticulate crest (Figs. 4-7).

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[2] In the more recent works, the order *Coniferales* is divided into six families of equal rank: *Abietaceæ*, *Taxodiaceæ*, *Cupressaceæ*, *Araucariaceæ*, *Podocarpaceæ* and *Taxaceæ* (See Chamberlain, 1935).

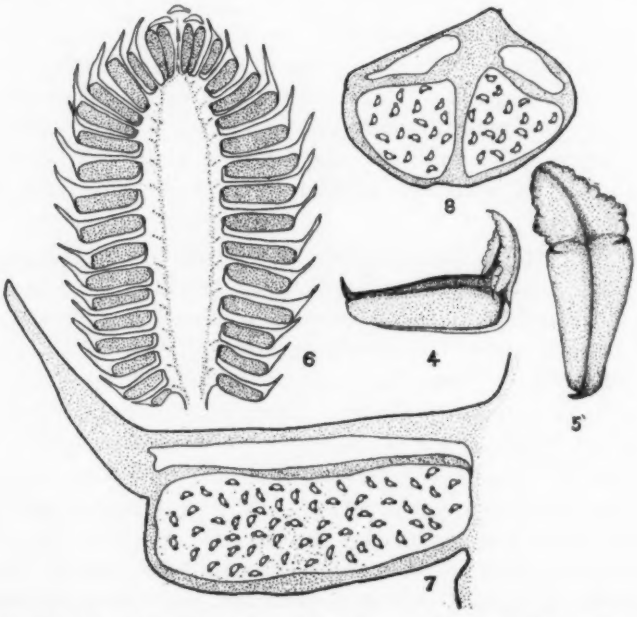
[3] I have preferred to use the terminology suggested by Maheshwari (1935).

[4] Figs. 2, 4 and 5 were very kindly prepared by my friend Mr. Bahadur Singh, M.Sc., to whom my best thanks are due.

The cones are initiated at both Simla and Mussoorie in June, and the pollen grains are shed from the middle of September to the first week of October. As there is no resting period and the entire development is completed in about three months, it does not seem necessary to give dates for all the stages. It frequently happens that different trees show cones of slightly different sizes and even the cones on the same tree differ to a certain extent in the degree of their development. Besides, even the same cone may show all the stages from the microspore mother cells to the formation of microspores. Chamberlain (1935, p. 288) mentions that "In *Pinus*, a section of the sporangium, in any direction, shows as wide a range of stages as are found from the top to the bottom of an anther of *Lilium*. In an extremely long microsporangium like that of *Araucaria*, stages at the top and bottom would probably be different."

4. *Early Development of the Microsporangium.*

The structure of the microsporangium agrees with that usually described for the other *Abietaceæ*. Fig. 6 shows the general structure of a male cone



FIGS. 4-8.—Figs. 4 and 5, Lateral and ventral views of a microsporophyll. × 8. Fig. 6, L.S. of a male cone (diagrammatic). × 4. Figs. 7 and 8, L.S. and T.S. of a microsporophyll. The pollen grains have been made much larger than they actually appear at this magnification. The empty spaces are the resin canals. ×17.

in longitudinal section ; Figs. 7 and 8 show the structure of a single microsporangium in longitudinal as well as transverse sections.

Fig. 3 shows a tranverse section of a very young microsporophyll. The two prominent groups of cells (stippled in the figure) represent the places of origin of the two microsporangia, with a large resin canal above each. Figs. 7 and 8 show resin canals in longitudinal and transverse sections of approximately mature sporangia. In his paper on *Larix leptolepis*, Doyle (1918 , Text-Fig. 2) has incidentally figured similar resin canals in *Cedrus atlantica*. The same thing is seen in *Larix* and *Pseudotsuga* (Doyle, 1918, pp. 312-313), but here the resin canals are found in a more distal part of the microsporophyll. In *Pseudolarix* they are much smaller, and in *Abies*, *Picea* and *Pinus* they are hardly distinguishable.

From this stage onwards all the cells of the microsporophyll—except the two sporogenous groups, the cells lining the resin canals, and a few cells around the vascular bundle—are full of a homogeneously staining resinous (?) substance which takes a red stain with safranin. It is probably this that hinders a rapid penetration of the killing fluids and prevents good fixation.

The wall of the microsporangium.—In the younger stages there is no sharp distinction between the cells of the archesporium and those of the wall layers. But very soon the former enlarge, the cytoplasm becomes denser and the nuclei attain greater prominence (Fig. 9), while the wall cells gradually become flattened and tabular. There is a total of four to five wall layers of which the one in immediate contact with the spore mother cells is the tapetum (Fig. 10).

As the microsporangium increases in size, the epidermis as well as one (Fig. 14) or two of the outermost wall layers become filled with a resinous substance and develop fibrous thickenings which are comparable to those, characteristic of the endothecium of angiosperms. Only at one place in the epidermis—as seen in tangential sections through the cone—the resinous contents and thickenings are absent. This plate of cells, running ventrally along the whole length of the sporangium, can be identified very early and determines the future line of dehiscence (Figs. 10 and 11).

The inner wall layers, lying between the hypodermis and the tapetum, cease to grow, and in consequence of the pressure caused by the enlargement of the sporangium, their cells become stretched and flattened and their nuclei and cytoplasm disorganise, so that even before the reduction divisions are over they present a crushed and structureless appearance (Fig. 11).

Tapetum.—During the earlier stages of development the tapetum cannot be distinguished from the adjacent cells. It is first clearly differentiated

when the mother cells are in the prophase of the first reduction division. Contradictory views have been expressed about its origin in the existing literature. Thus, Ferguson (1904), Sethi (1928), Mathews (1932) and several other authors have concluded that it is formed by the sterilisation of the outer layer of the sporogenous tissue. Chamberlain (1935, p. 285), on the contrary, writes in his recent book that it has been shown to be derived from the wall layers in so many cases that any claim of a sporogenous origin would have to be strongly supported. In spite of a very careful examination of my preparations, I could not succeed in tracing its origin with certainty, but I get the impression that it arises in the former way, *i.e.*, from the outermost layer of the sporogenous tissue. To the best of my knowledge, the only gymnosperm in which it has been clearly traced to have a parietal origin is *Ephedra foliata* (Maheshwari, 1935).

At the time of the reduction divisions of the pollen mother cells, the tapetal cells increase in size, the cytoplasm becomes vacuolate and each cell becomes bi-nucleate through mitotic division of the original nucleus (Figs. 11 and 12).

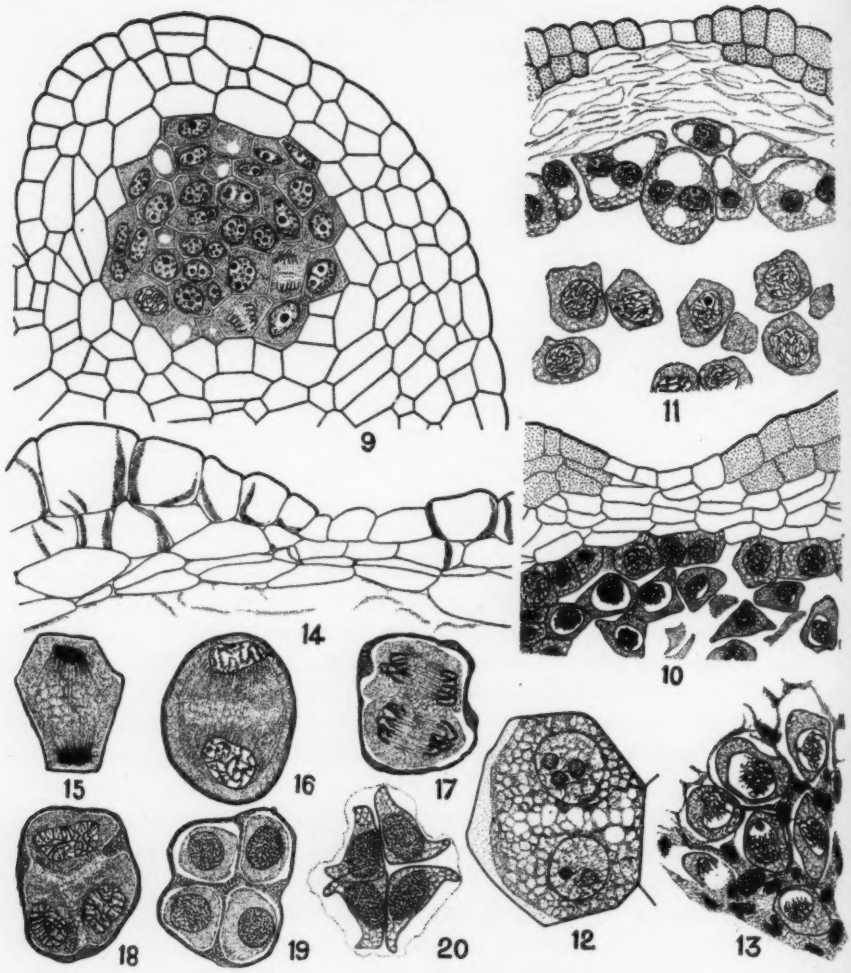
At the tetrad stage, the tapetal protoplasts were observed to protrude inside the pollen sac in some of the sporangia. This is comparable to the condition reported by Luxemburgowa (1922) in *Thuja orientalis* and *T. occidentalis*. In one or two cases, cytoplasmic masses which could not have been derived except by the fusion of the tapetal protoplasts, were seen in between the dividing mother cells (Fig. 13). The only other Conifer reported to have a tapetal periplasmodium is *Larix* (Luxemburgowa, 1922; Woycicki, 1931).

Degeneration of the tapetal cells begins after the reduction divisions are over and they finally disappear when the pollen grains have reached maturity.

5. *Microsporogenesis.*

The number of microspore mother cells inside a microsporangium is estimated to be no less than 2,500. At the beginning of the reduction divisions the mother cells separate from one another and become rounded at the corners. After the usual synizesis and diakinesis stages the nuclear membrane disappears. The fibres of the heterotypic spindle are extremely thin and delicate (Fig. 15). A cell plate was never observed after the telophase, but a lighter and more vacuolated area of cytoplasm is invariably seen in the equatorial region in slides stained with iron-alum hæmatoxylin (Fig. 16).

The two daughter nuclei formed after the first reduction division immediately enter upon the second without undergoing any appreciable



FIGS. 9-20.—Fig. 9, Part of a young microsporangium showing two to three wall layers below the epidermis and a group of sporogenous cells. × 500. Fig. 10, Same, more advanced stage showing well differentiated tapetum and microspore mother cells in synizesis. The dotted cells in the wall layers are filled with resin; the empty cells indicate the place of opening of the sporangium. × 500. Fig. 11, Same, still more advanced stage. The middle layers have degenerated and the tapetal cells have become two-nucleate. × 500. Fig. 12, An enlarged tapetal cell. × 1000. Fig. 13, Part of a microsporangium showing microspore mother cells imbedded in a tapetal periplasmodium. × 500. Fig. 14, Part of the wall of a mature microsporangium showing thickenings in the epidermis and hypodermis. Dehiscence occurs at the unthickened part. × 500. Fig. 15, Microspore mother cell; telophase of the heterotypic division. × 1000. Fig. 16, Same,

in interkinesis. × 1000. Fig. 17, Homotypic division, early telophase. × 1000. Fig. 18, Homotypic division completed, beginning of constriction furrows at the periphery between each of the four daughter nuclei. × 1000. Fig. 19, Tetrad of microspores separated by a gelatinous matrix. × 1000. Fig. 20, Young microsphores; the swollen wall has been nearly absorbed. × 1000.

period of rest. The two spindles may be arranged in the same direction or at right angles to one another. No cell plates are laid down after the termination of this division also, although a vacuolar lightly staining area could be made out in between each of the four daughter nuclei (Fig. 18).

In *Pinus strobus*, Miss Ferguson (1904) denies the presence of any cell plate formation after the heterotypic division, but she does not mention whether a cell plate is laid down or not even after the homotypic division. Sethi (1928) states that evanescent cell plates are laid down in *Pinus longifolia* after both the divisions. Saxton (1929) reports that a permanent wall is sometimes formed after the heterotypic division in *Larix europæa*. According to Mathews (1932) a cell plate is seen at this stage (after the heterotypic division) in *Pinus palustris* also, but he could not decide whether it was ephemeral or permanent. In spite of a careful examination of several slides I was unable to see any trace of the cell plates in *Cedrus deodara*. At the termination of the homotypic division the four microspore nuclei are connected with one another by means of spindle fibres.

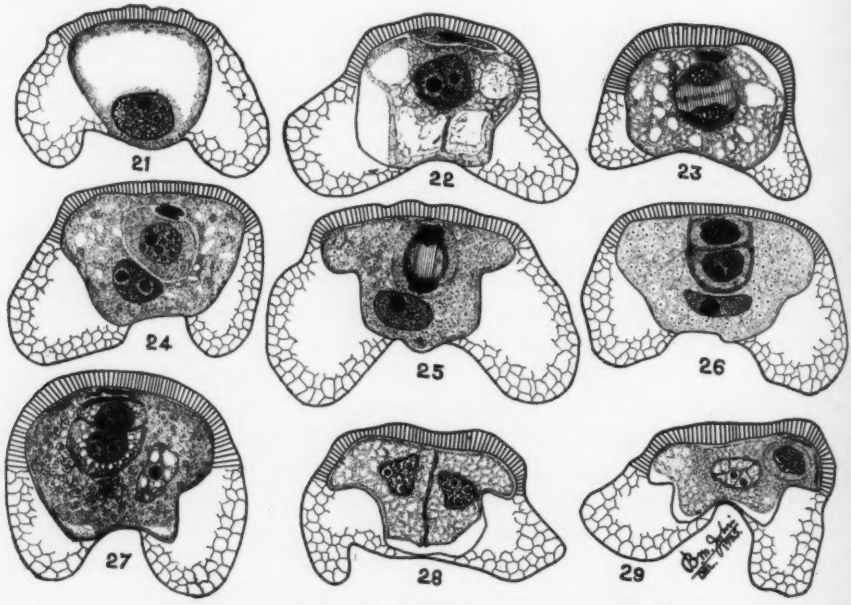
When the mother cells are undergoing reduction divisions, their walls swell and attain a remarkable thickness as seen in slides treated with Fast Green or stained in Delafield's hæmatoxylin, but it could not be determined whether this is due to a special secretion from the cytoplasm or a swelling of the original cell wall (Fig. 17). Quadripartition occurs by means of constriction furrows which continue to grow centripetally followed by ingrowths of the swollen wall (Fig. 18). The resulting microspores now seem to be embedded in a homogeneous jelly-like matrix which stains lightly with Fast Green but is hardly noticeable in sections stained with Iron-alum hæmatoxylin (Fig. 19).

Finally the remnants of the mother cell wall disappear altogether (Fig. 20), and the young microspores are set free. Ferguson (1904) reported that this is due to a rupture of the wall, while Sethi (1928, p. 132) is of opinion that in *Pinus longifolia* the microspores "come out through definite openings appearing in the outer walls of the mother cell". Mathews (1932), on the other hand, remarks that in *P. palustris* the wall is absorbed by some digestive process. My observations are in agreement with those of Mathews.

6. *Male Gametophyte.*

The young microspores have a comparatively larger size than those of *Pinus*. The wall soon differentiates into an exine and an intine. The two separate from each other at two opposite points giving rise to the wings. After the escape of the microspores from the mother cell wall, the exine becomes beautifully marked by the formation of delicate ridges.

The nucleus of the microspore (Fig. 21) leaves its central position and moves upwards. The first division cuts off a very small prothallial cell (Fig. 22). Soon after, a second prothallial cell is laid down against the first. Both the prothallial cells are ephemeral and in the mature pollen grains they can be seen only in favourable sections (compare Figs. 23, 24, 27 with Figs. 25 and 26).



FIGS. 21-29.— ×600. Fig. 21, A uni-nucleate pollen grain. Fig. 22, Pollen grain with the first prothallial cell cut off. Fig. 23, Two prothallial cells; the tube and antheridial nuclei just formed. Fig. 24, Same, antheridial cell well organised. Fig. 25, Division of the antheridial cell; prothallial cells not visible. Fig. 26, Antheridial cell divided into a sterile and a spermatogenous cell. The nucleus of the sterile cell has assumed an irregular appearance and gives an impression as if it is going to fragment. Fig. 27, A mature pollen grain with two prothallial cells, sterile cell, spermatogenous cell and tube nucleus. Fig. 28, A double pollen grain. Fig. 29, Another abnormal pollen grain with the prothallial cells and the antheridial cell displaced to one corner and the tube nucleus lying in the centre.

A third mitosis now follows resulting in the production of the tube and antheridial nuclei. An ephemeral cell plate is laid down on this spindle, but it soon disappears (Fig. 23). The cytoplasm surrounding the antheridial cell becomes denser than the general cytoplasm of the rest of the pollen grain, which contains numerous starch grains and vacuoles.

The last division concerns only the antheridial cell which gives rise to a sterile cell (stalk cell) and the spermatogenous cell (body cell) (Figs. 25-27). At this stage the pollen grains are shed. The division of the spermatogenous cell must be taking place on the nucellus.

The tube nucleus takes a very light stain with hæmatoxylin and becomes irregular and amœboid in outline. Occasionally it is seen to have shifted from its central position to a lateral one (Fig. 27). The nucleus of the sterile cell also shows a slightly irregular appearance and in one case it seemed as if it was going to fragment into two along its whole length (Fig. 26). Hutchinson (1914) found that in *Abies* the nucleus of the sterile cell sometimes has an appearance which is suggestive of amitosis, while in others it had actually divided to give rise to two cells.

Degenerations.—Degenerations are met with quite commonly from the mother cell stage onwards. Only a few or all the mother cells in a sporangium may degenerate. The fact, that in a few cases some degenerating mother cells were found side by side with young microspores in the same sporangium, indicates that not all of them may go through the reduction divisions. A similar case was reported by Lang (1897) in *Stangeria paradoxa*. The microsporangia at the base of the cone and sometines even at the top are almost abortive. During degeneration the nuclei take a uniformly black stain but the cytoplasm stains very poorly. Occasionally one may find a healthy tapetum enclosing shrunken and degenerated mother cells or tetrads.

Abnormal pollen grains.—Several double pollen grains were seen (Fig. 28), but I am not in a position to state definitely as to how such a condition originates. Double pollen grains have also been reported in *Picea* (Pollock, 1906; Hutchinson, 1915) and *Larix* (Coker, 1904; Doyle, 1918), but the statements about their origin are conflicting. According to Coker the pollen mother cell occasionally divides into two cells which fail to separate and thus a double pollen grain originates. According to Hutchinson the first spindle of the microspore nucleus twists through a right angle, the resulting wall running straight across the cell and dividing it into two. From his work on *Larix leptolepis*, Doyle (1918) considers the latter method to be the more probable of the two.

Fig. 29 shows another abnormal pollen grain where the microspore nucleus seems to have shifted to a lateral position before cutting off the first prothallial cell. The second prothallial cell was not cut off in the same position but at right angles to the first. The antheridial cell lies in a corner and the tube nucleus in the centre of the pollen grain.

7. *Summary.*

(1) At Simla and Mussoorie the male cones are initiated in June and the pollen is shed from the middle of September to the first week of October. Each is a solitary, terminal spike with numerous spirally arranged microsporophylls.

(2) Two microsporangia are situated on the abaxial side of the sporophyll which terminates into a denticulate crest. Above each sporangium and along its whole length runs a resin canal.

(3) The archesporium is hypodermal in origin. There are four to five wall layers of which only the epidermis and one or two layers below it persist in the mature microsporangium. All of their cells acquire fibrous thickenings except those forming the mid-ventral line, which marks the place of dehiscence.

(4) The tapetum is derived from the layer of cells in immediate contact with the sporogenous tissue and the cells become bi-nucleate through mitotic division of the original nuclei. In rare cases a tapetal periplasmodium is also formed.

(5) The reduction divisions are simultaneous. Cytokinesis occurs through furrows. The microspores acquire wings while still enclosed in the mother cell wall.

(6) The microspore nucleus divides to produce two ephemeral prothallial cells, a sterile cell, the spermatogenous cell and the tube nucleus. Starch grains are abundant in the pollen grains.

(7) Degenerations are quite common and may involve one to several entire sporangia in a cone or at times only a few cells in a sporangium.

(8) Double pollen grains are met with as an abnormality.

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INVESTIGATIONS ON THE ROLE OF ORGANIC MATTER IN PLANT NUTRITION.

Part XI. Effect of Manuring on the Growth and Intake of Silicon by Dry and Wet Cultivated Rice.

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It has been shown in some previous communications (Sreenivasan and Subrahmanyan, 1934 ; Bhaskaran *et al.*, 1934 ; Sundara Iyengar and Subrahmanyan, 1935) that decomposition of organic matter under conditions of swamp soil results in increased dissolution of minerals. Attention has also been drawn (Sreenivasan, 1934) to the high silicon content of the rice plant and its probable bearing on the physiology of that crop. Since the rice plant flourishes best under the conditions of the swamp soil and responds well to organic manures, it appeared probable that those two conditions are particularly favourable to both dissolution and intake of silicon by that crop. With a view to throwing further light on that aspect of the problem, the present enquiry was undertaken.

Experimental.

A number of glazed earthenware pots were prepared in the usual way, a clay soil (42 lbs.) from an adjoining area being used for filling. The treatments were as follows.—(*a*) unmanured and maintained at 60 per cent. saturation with water ; (*b*) unmanured and maintained in a puddled condition ; (*c*) manured with leaves of *Pongamia glabra* at 100 g. per pot and maintained as in (*a*) ; (*d*) manured as in (*c*) and maintained as in (*b*) ; (*e*) manured with seed-cake of *Pongamia glabra* at 70 g. per pot and maintained as in (*a*) and (*f*) manured as in (*e*) and maintained as in (*b*). Eighteen pots were allotted for each treatment. The green manure was applied in the shape of fresh, tender leaves cut into small bits while the cake was added as fine powder. In either case the manure was mixed thoroughly with the soil to a depth of about 6 inches.

The pots in series (*b*), (*d*) and (*f*) were made as above on 3rd July 1934 while those in series (*a*), (*c*) and (*e*) were made on 12th July 1934. All the pots were kept in the open and maintained under conditions which would correspond to those obtaining in the field. The drainage was rather slow in the puddled series (*b*, *d* and *f*), while the other sets of pots drained quite freely.

TABLE I.

Meteorological Observations.

Week ending.....	July				August				September					October			
	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27
Maximum Temp.° (weekly averages) ..	80.2	84.7	88.0	84.1	78.0	82.4	80.6	83.5	84.6	84.6	86.8	86.1	87.8	88.8	82.0	83.0	87.7
Minimum Temp.° (weekly averages) ..	66.9	66.0	67.4	67.6	66.1	67.4	66.9	65.7	66.5	66.7	66.8	65.4	65.8	68.4	67.0	66.7	66.7
Total sunshine (from week to week, hrs.)	10.6	33.8	44.9	20.5	5.1	27.5	13.9	41.3	41.2	30.6	46.9	39.3	53.6	46.7	20.8	46.5	39.5
Total rainfall (from week to week, inches) ..	0.70	..	..	0.06	1.31	0.35	0.65	0.69	0.21	0.05	0.08	0.05	0.15	0.24	5.03	0.22	0.27
Relative humidity (weekly averages) ..	75	64	61	68	83	72	76	70	66	63	61	62	56	59	81	67	65

Paddy (Var., Adt., 3—a four month crop) was sown in separate seed pots on 12th June 1934. The seedlings were transplanted into the experimental pots on 16th July 1934 when they had attained an average height of about 6 inches. Four pairs of seedlings were transferred to each pot, the planting being done two together. When the seedlings had all become well-established in the soil (about a week after transplanting) enough water was added to the pots in series (*b*), (*d*) and (*f*) so as to keep the soil well under submergence (about 3 inches) and slow drainage allowed to take place. Water was added at frequent intervals to keep the level of submergence nearly constant. This was continued till the first week of October when grains began to form in all the cases. Pots in series (*a*), (*b*) and (*e*) were just kept moist by daily addition of water which was also stopped in the beginning of October.

Meteorological Observations.—The growing period was from 16–7–1934 to 26–10–1934; during this period systematic daily records of maximum and minimum temperature, hours of sunshine, rainfall and relative humidity were maintained. The weekly averages have been presented in Table I.

The season corresponded to the active part of the south-west monsoon and was, therefore, attended by a fair amount of rain, especially during the last stages. The majority of the days were, however, fairly bright and sunny and moderately warm. Watering was not done during periods of heavy rainfall and, at the time of harvest, the pots were practically dry.

Samples for Analysis.—At different stages of development, representative samples (8 for each treatment) of whole plant were taken by careful uprooting. They were then taken to the laboratory where they were carefully washed in running water and dried between folds of filter paper. After making the necessary determinations on the green material, the plants were divided into roots and tops and the corresponding fresh and oven-dry (100°) weights determined.

The dates of sampling at different developmental stages of the plant are given in Table II.

It was observed that the rate of growth was not the same in all the cases, being obviously due to the different treatments. Particularly was this noticeable in the case of series (*f*) treated with hongay cake and swamped, wherein there was an initial set-back, the tillers appearing long after they had done so in the other cases. This was perhaps due to the period of puddling having been insufficient and consequent persistence of toxicity for some time after transplantation (Onodera, 1923). This set-back was however only temporary: all the plants recovered fully after some time, and, indeed, as shown elsewhere, the best yields were obtained from that series.

TABLE II.

Date of sampling	Age of plant since germination (18-6-1934) days	Developmental stage (approximate)
16—7—1934	28	Seedlings (Transplantation stage)
15—8—1934	58	Beginning to tiller (Green stage)
18—9—1934	92	Mature plants (Stage of active tillering)
28—9—1934	102	Just flowering (Bloom stage)
7—10—1934	111	Panicles were out, but seeds only partially formed (Grain formation stage)
26—10—1934	130	Seeds fully formed and ripe (Harvesting stage)

Ash and Silica.—The roots were cut into very small bits while the tops were ground to pass the 40-mesh sieve. A known weight of the material in a platinum dish was first heated over a low flame and then ignited at dull red heat in a muffle furnace till almost white after which the ash weight was determined. Silica in the ash was estimated from the loss in weight after volatilisation of same with hydrofluoric acid (*A. O. A. C.*, 1930).

It was observed that in the case of root ash, a considerable amount of brick red residue was always left behind after volatilisation of silica. This was probably finely divided iron oxide which, on account of its having been strongly ignited, was not completely soluble in concentrated hydrochloric acid.

Analytical Results: Growth data.—It will be seen from a comparative study of the different tables (Tables III—VIII) that the best growth was obtained in both the manured and swamped series while the manured "dry" series came next and control (unmanured) series last. The unmanured (control) "dry" series showed particularly poor growth and root development and the plants began to dry up and even die in a few cases before the completion of the experiment. The rate of development of the plants, the roots and the total dry weight were all also in the same order as above except, as mentioned before, for an initial lag in the case of series (*f*) (cake swamped). There was steady increase in the dry weight upto the 111th day after which there was fall in all the cases. This was due to the fact that after that period

TABLE III.

Manure : Hongay Leaves—Swamped.

(*Series* d)

Age of plants (in days)	TOPS				ROOTS			Total dry weight of plant in g.	Ratio Tops / Roots
	Tillers Number per plant	Height in cm.	Fresh weight in g.	Dry weight in g.	Length in cm.	Fresh weight in g.	Dry weight in g.		
(1)	(2)*	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
28	..	19	0·60	0·11	13	0·65	0·18	0·29	0·64
58	3.3 (–)	55	10.84	2·25	24	1·33	0·36	2·61	6·2
92	6·5 (2.6)	82	13·75	3·39	38	2·59	0·61	4·00	5·4
102	8.3 (4.9)	85	18·41	5·00	41	2·18	0·61	5·61	8·2
111	10·3 (8·8)	97	22 93	6·43	44	3·30	0·85	7·28	7·6
130	8.9 (8.0)	89	22·23	6·23	36	3·25	0·78	7·01	13·7

(*Figures in columns 2, 4, 5, 7, 8 and 9 represent the average of 16 plants in each series. In column 2, the bracketted figures indicate the number of earing tillers.)

TABLE IV.

Manure : Hongay Cake—Swamped.

(*Series* f)

Age of plants (in days)	TOPS				ROOTS			Total weight of plant in g.	Ratio Tops / Roots
	Tillers Number per plant	Height in cm.	Fresh weight in g.	Dry weight in g.	Length in cm.	Fresh weight in g.	Dry weight in g.		
(1)	(2)*	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
28	..	19	0·60	0·11	13	0·65	0·18	0·29	0·64
58	2·0 (–)	39	7·15	1·49	19	0·80	0·21	1·70	7·0
92	3·9 (–)	58	7·83	1·89	31	1·69	0·41	2·30	4·6
102	6·8 (1·5)	62	9·98	2·40	38	..	..	..	..
111	8·9 (8·0)	86	21·34	5·84	39	3·19	0·76	6·60	7·7
130	9·9 (9·0)	80	19·55	5·65	39	2·63	0·76	6·41	15·4

(*In column 2, first figure indicates all the tillers in one plant while the bracketted figures are the number of earing tillers.)

TABLE V.

Manure: Hongay Cake—'Dry'

(*Series* e)

Age of plants (in days)	TOPS				ROOTS			Total dry weight of plant in g.	Ratio Tops / Roots
	Tillers Number per plant	Height in cm.	Fresh weight in g.	Dry weight in g.	Length in cm.	Fresh weight in g.	Dry weight in g.		
(1)	(2)*	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
28	..	19	0·60	0·11	13	0·65	0·18	0·29	0·64
58	2·4 (–)	48	10·13	2·16	23	1·30	0·31	2·47	7·0
92	3·4 (1·4)	66	13·80	2·98	26	1·46	0·38	3·36	7·9
102	5·8 (2·8)	75	12·63	3·89	20	2·30	0·65	4·54	6·0
111	5·5 (4·8)	88	12·08	3·63	21	2·88	0·95	4·58	3·8
130	4·5 (3·8)	71	8·58	4·04	24	2·09	0·63	4·67	6·5

(*In column 2, first figure indicates all the tillers in one plant while the bracketted figures are the number of earing tillers.)

TABLE VI.

Manure: Hongay Leaves—'Dry'

(*Series* c)

Age of plants (in days)	TOPS				ROOTS			Total dry weight of plant in g.	Ratio Tops / Roots
	Tillers Number per plant	Height in cm.	Fresh weight in g.	Dry weight in g.	Length in cm.	Fresh weight in g.	Dry weight in g.		
(1)	(2)*	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
28	..	19	0·60	0·11	13	0·65	0·18	0·29	0·64
58	3·0 (–)	46	9·74	2·39	21	1·15	0·35	2·74	6·8
92	4·9 (1·4)	69	12·38	3·48	29	1·08	0·33	3·81	10·7
102	5·3 (2·3)	71	11·58	3·08	24	2·13	0·69	3·77	4·5
111	4·9 (4·0)	77	10·03	3·08	28	2·15	0·64	3·72	4·8
130	5·1 (3·3)	69	7·28	2·90	23	2·45	0·61	3·51	4·7

(*In column 2, first figure indicates all the tillers in one plant while the bracketted figures are the number of earing tillers.)

TABLE VII.

Unmanured (Control)—Swamped.

(*Series* b)

Age of plants (in days)	TOPS				ROOTS			Total dry weight of plant in g.	Ratio Tops / Roots
	Tillers Number per plant	Height in cm.	Fresh weight in g.	Dry weight in g.	Length in cm.	Fresh weight in g.	Dry weight in g.		
(1)	(2)*	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
28	..	19	0·60	0·11	13	0·65	0·18	0·29	0·64
58	2·5 (–)	42	6·69	1·64	18	1·08	0·26	1·90	6·0
92	4·1 (0·9)	63	7·94	2·09	29	2·10	0·56	2·65	3·7
102	4·0 (1·6)	74	9·65	2·68	25	1·93	0·51	3·19	5·3
111	3·8 (3·0)	77	8·19	2·19	24	1·98	0·56	2·75	3·9
130	3·6 (1·4)	64	5·78	4·44	22	1·38	0·41	4·85	5·0

(*In column 2, first figure indicates all the tillers in one plant while the bracketted figures are the number of earing tillers.)

TABLE VIII.

Unmanured (Control)—'Dry'.

(*Series* a)

Age of plants (in days)	TOPS				ROOTS			Total weight of plant in g.	Ratio Tops / Roots
	Tillers Number per plant	Height in cm.	Fresh weight in g.	Dry weight in g.	Length in cm.	Fresh weight in g.	Dry weight in g.		
(1)	(2)*	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
28	..	19	0·60	0·11	13	0·65	0·18	0·29	0·64
58	2·3 (–)	34	2·56	0·60	14	0·75	0·18	0·78	3·3
92	3·0 (0·8)	48	3·00	0·95	15	0·53	0·13	1·08	7·6
102	3·3 (1·1)	51	3·38	0·91	12	0·50	0·13	1·04	7·3
111	2·4 (2·0)	70	3·48	0·99	19	0·70	0·18	1·17	5·7
130	1·5 (0·8)	65	3·04	1·40	18	0·79	0·63	1·63	6·2

(*In column 2, first figure indicates all the tillers in one plant while the bracketted figures are the number of earing tillers).

a few of the leaves had dried up and withered and even dropped out sometimes. Variations in the moisture content of the roots were not very significant while in the case of the tops, it was noticed that there was a gradual rise in the percentage dry matter till the stage of grain formation after which it was practically constant (about 30 per cent.) in all the cases.

The results show in a striking manner how the wet soil conditions are favourable at all stages for the growth of the rice plant. Thus, in the manured pots the weight of straw in the dry series at any stage was often less than half what was obtained in the case of the corresponding wet treated series ; while with regard to the roots, not only was the extent of root development greater in the case of the swamped and manured series but also the area over which the roots spread was very much more in those cases than in the "dry" manured ones.

Yields.—The yields of straw and of grain at harvesting stage in each of the series is given in Table IX. Results represent the average yield per plant and is the average of 48 plants in each case.

TABLE IX.

Yield (dry weight in g. per plant) at harvesting.

	Green manured swamped	Hongay cake swamped	Unmanured swamped	Green manured 'dry'	Hongay cake 'dry'	Unmanured 'dry'
Straw weight	6·45	5·61	1·55	1·78	1·86	0·88
Grain weight	4·20	3·58	0·45	1·05	1·21	0·35
$\frac{\text{Grain}}{\text{Straw}} \times 100$	63·6	63·7	29·0	59·2	29·0	39·1

It will be seen that the yield of straw in the case of dry series is only about 30-35 per cent. of that in the swamped series while the corresponding ratio in the yield of grain is also about 25-30 per cent. The percentage of moisture in the straw grown under wet conditions was slightly higher than in the other cases while it was almost constant throughout in the case of the grains.

Ash Analyses.—Tables X-XV give the ash analyses of the various parts of the plant at different stages. The percentage of ash or of silica in the roots varies irregularly in all the series and does not seem to be of any significance. Even the percentage of silica in the ash varies somewhat

TABLE X.

Green Manured—Swamped.

(*Series* d)

Material analysed	Age of material (in days)	Per cent. dry matter in green sample	Per cent. on dry matter of		Per cent. in ash of silica	Total intake of silica per plant (in mg. average of 16 plants)
			Total ash	Silica		
Roots	28	26·9	11·60	4·68	40·33	8·2
	58	27·4	10·02	4·10	40·93	14·9
	92	23·7	9·84	4·12	41·87	25·2
	102	28·2	13·59	5·60	41·19	34·3
	111	25·8	10·90	5·12	46·98	43·5
	130	23·8	9·40	4·02	42·77	31·1
Whole Plant above Ground	28	18·8	15·22	8·97	58·93	10·1
	58	20·8	17·04	10·40	61·03	234·0
	92	24·6	20·37	13·33	65·43	450·8
	102	27·2	19·52	12·91	66·15	645·5
	111	28·1	16·04	10·16	63·33	652·8
	130	28·0	16·05	10·60	66·04	660·3
Green Leaves and Stalks	111	29·5	19·66	12·10	61·55	
	130	26·4	17·80	10·19	57·23	
Panicles (immature)	(111)	54·3	15·67	13·29	84·82	
do. (with ripe seed)	(130)	83·7	11·24	8·54	76·00	

TABLE XI.

Hongay Cake—Swamped.

(*Series* f)

Material analysed	Age of material (in days)	Per cent. dry matter in green sample	Per cent. on dry matter of		Per cent. in ash of silica	Total intake of silica per plant (in mg.) average of 16 plants)
			Total ash	Silica		
Roots	28	26·9	11·60	4·68	40·33	8·2
	58	26·6	10·46	4·06	39·72	8·6
	92	24·4	10·42	4·42	42·42	18·2
	102	..	11·87	4·54	38·20	..
	111	23·9	12·01	5·20	43·33	39·7
	130	29·0	9·65	4·14	42·90	31·6
Whole Plant above Ground	28	18·8	15·22	8·97	58·93	10·1
	58	20·8	16·47	9·90	60·08	147·5
	92	24·2	20·30	12·71	62·62	239·9
	102	24·7	20·11	13·62	67·72	326·8
	111	27·4	17·40	11·22	66·01	655·0
	130	28·9	16·55	10·80	65·25	610·1
Green Leaves and Stalks	111	27·6	18·42	10·86	58·96	
	130	32·4	17·03	11·22	65·91	
Panicles (immature)	111	51·5	17·01	14·56	85·63	
do. (ripe)	130	82·1	9·12	7·15	78·39	

TABLE XII.

Green Manured—'Dry'

(*Series* c)

Material analysed	Age of material (in days)	Per cent. dry matter in green sample	Per cent. on dry matter of		Per. cent in ash of silica	Total intake of silica per plant (in mg. average of 16 plants)
			Total ash	Silica		
Roots	28	26·9	11·60	4·68	40·33	8·2
	58	30·4	11·02	3·92	35·57	13·7
	92	30·2	9·96	3·95	39·69	12·8
	102	32·4	9·92	3·96	39·92	27·2
	111	29·7	10·41	4·52	43·41	28·8
	130	25·0	9·01	3·74	41·52	22·9
Whole Plant above Ground	28	18·8	15·22	8·97	58·93	10·1
	58	24·6	16·98	10·12	59·58	241·5
	92	28·1	17·27	10·64	61·63	369·8
	102	26·5	17·60	11·23	63·78	345·1
	111	30·7	17·68	11·62	65·73	357·4
	130	39·9	17·29	10·65	61·60	308·9
Green Leaves and Stalks	111	30·8	18·64	10·68	60·01	
	130	29·5	17·96	10·40	57·89	
Panicles (immature)	111	53·7	16·22	14·04	86·56	
do. (ripe)	130	85·5	10·56	8·01	75·84	

TABLE XIII.

Hongay Cake—'Dry'.

(*Series* e)

Material analysed	Age of material (in days)	Per cent. dry matter in green sample	Per cent. on dry matter of		Per cent. in ash of silica	Total intake of silica per plant (in mg. average of 16 plants)
			Total ash	Silica		
Roots	28	26·9	11·60	4·68	40·33	8·2
	58	23·7	9·24	4·21	45·56	13·2
	92	25·8	9·87	3·86	39·11	14·5
	102	28·5	9·70	4·82	49·68	31·7
	111	33·0	10·06	4·54	45·14	43·1
	130	29·9	8·94	3·99	44·64	24·9
Whole Plant above Ground	28	18·8	15·22	8·97	58·93	10·1
	58	21·4	15·86	9·68	61·02	209·4
	92	21·6	17·58	11·26	64·03	336·5
	102	30·8	19·14	10·24	53·51	398·0
	111	30·0	16·81	10·80	64·24	391·4
	130	47·1	17·02	9·84	57·81	..
Green Leaves and Stalks	111	33·3	18·09	10·04	55·5	
	130	29·9	..	..	..	
Panicles (immature)	111	51·9	16·03	14·10	87·96	
do. (fully ripe)	130	79·8	9·94	7·57	76·15	

TABLE XIV.

Unmanured—Swamped.

(*Series* b)

Material analysed	Age of material (in days)	Per cent. dry matter in green sample	Per cent. on dry matter of		Per cent. in ash of silica	Total intake of silica per plant (in mg. average of 16 plants)
			Total ash	Silica		
Roots	28	26·9	11·60	4·68	40·33	8·2
	58	25·0	10·10	..	..	..
	92	27·0	9·76	4·12	42·22	23·2
	102	26·3	10·90	4·67	42·85	23·9
	111	28·5	9·10	4·96	54·51	27·9
	130	29·6	9·66	4·42	45·75	18·2
Whole Plant above Ground	28	18·8	15·22	8·97	58·93	10·1
	58	24·5	15·38	8·94	58·13	146·6
	92	26·4	17·63	10·96	62·19	229·0
	102	27·7	16·55	10·10	61·02	270·4
	111	26·7	16·24	10·86	66·91	238·4
	130	35·5	16·64	11·02	66·22	226·3
Leaves and Stalks	111	30·5	16·84	9·48	56·28	
	130	29·7	17·62	10·13	56·96	
Panicles	111	52·2	15·92	12·60	79·14	
do. (ripe)	130	83·1	11·24	8·26	73·50	

TABLE XV.

Unmanured—'Dry'.

(*Series* a)

Material analysed	Age of material (in days)	Per cent. dry matter in green sample	Per cent. on dry matter of		Per cent. in ash of silica	Total intake of silica per plant (in mg. average of 16 plants)
			Total ash	Silica		
Roots	28	26·9	11·60	4·68	40·33	8·2
	58	23·8	11·20	..	..	..
	92	24·0	9·48	4·00	42·20	5·0
	102	25·0	10·45	4·80	45·93	6·0
	111	24·7	8·96	4·23	47·21	7·4
	130	28·6	9·02	3·68	40·80	8·3
Whole Plant above Ground	28	18·8	15·22	8·97	58·93	10·1
	58	23·2	16·49	9·98	60·50	59·9
	92	31·7	17·11	11·44	66·86	108·6
	102	27·1	18·33	13·34	72·78	121·9
	111	28·3	17·18	11·82	68·84	116·8
	130	46·1	16·70	10·96	65·63	121·9
Leaves and Stalks	111	30·2	17·34	10·92	62·98	
	130	31·5	16·90	10·93	64·68	
Panicles	111	53·1	15·92	13·21	82·96	
do. (ripe)	130	81·3	13·20	9·87	74·76	

irregularly although it might be said that it tends to increase in the later stages of growth. The total intake of silicon by the roots increases with time. Silica is assimilated in quite large quantities by roots of plants grown under swamped conditions.

The variations in the ash content of the tops are not, however, so irregular as with the roots. There is gradual rise in the percentage of the ash during the first 102 days after which this falls to about the same value in all the cases. The rate of rise is greater in the case of series (*d*) and (*f*), somewhat less with (*c*) and (*e*) and least in the unmanured series (*a*) and (*b*). More or less similar variations may be said to take place in regard to the percentage of silicon in the dry matter.

The percentage of silica in the ash has been calculated and given separately as it was thought that the percentage of the various ash constituents in the dry matter is less significant on account of possible fluctuations in the total ash. These figures would show that, as the plant ages, there is a steady increase in the percentage of silica up to the period of grain formation after which it remains fairly constant. It is of interest to note that while silica in the dry matter decreases after the 102nd day it remains about the same in the ash of the dry matter after this date. This is due to the fact that in the former case the percentage of silica in the dry matter of the grains is less than in the straw while the reverse is true with regard to the composition of the ash itself.

The foregoing sets of results would indicate that paddy grown under swamped conditions appears to be very much richer in silicon than under other conditions. In fact, the total intake of silica as calculated for the different series at different stages is far more in these cases than in the others. Manured "dry" series comes next in order with regard to silica uptake while the unmanured ones rank last. The total absorption of silica attains a maximum by about the 102nd day after which it remains practically the same in each series for the rest of the life period of the crop.

With a view to finding the distribution of silica between the green leaves and stalks and the grains, the ash and silica analyses on the above ground portion of the plant was done separately in the grains and in the straw on separate samples taken on the 111th and 130th day. The grains were all immature on the first day while, on the second sampling day, they were fully ripe. The results (included in Tables X–XV) indicate that the silica in the ash of the leaves is practically the same at both the periods of sampling in all the cases, though they were somewhat less than the silica content of the leaves of corresponding samples taken on the 102nd day.

The ash of the panicles on the other hand was considerably richer in silica while the grains were yet unripe than when they were fully formed and ripe. The results would thus suggest that (*a*) the absorption of silicon from the soil ceases after the flowering stage, (*b*) there is a transference of silicon from the green leaves and stalks to the panicles during the stage of grain formation, and (*c*) during the ripening of the grain only the constituents of the grain other than silicon are transported from the stem and stalk.

Absorption of Other Minerals by the Plant.—Table XVI gives the total intake of minerals other than silicon by the plant (above ground portion) at the different stages. It would be seen that the absorption of minerals as of silicon is greatest in the swamped manured series, less with the "dry" manured series and least in the case of the unmanured series. Absorption increases as the plant ages but after the flowering period, that is, after about 102 days, the plant does not seem to take any more minerals from the soil.

TABLE XVI.

Absorption of Mineral Constituents other than Silicon.

Age of plant in days	Green manured swamped	Hongay cake swamped	Unmanured swamped	Hongay leaf 'dry'	Hongay cake 'dry'	Unmanured 'dry'
	Intake of minerals (in mg.) per plant (averages)					
28	7·04	..	..	..	..	..
58	149·4	97·8	105·5	163·8	243·8	39·0
92	238·5	143·3	139·3	230·4	175·5	53·9
102	330·5	155·8	172·5	195·9	346·0	45·5
111	377·9	366·6	117·9	186·4	218·6	53·0
130	345·5	324·8	115·1	192·6	290·0	80·4

Discussion.

The present enquiry has shown that for optimum growth, the rice plant requires swamp soil conditions and that, under such conditions, it absorbs considerable quantities of silica from the soil system. The latter observation would mean that under swamp conditions more silica is rendered soluble in the soil or available to the plant. In order to verify whether there is increased dissolution of silica under swamped soil conditions, attempts were made to follow the amount of soluble silica present in the soil system in the

different series afore-mentioned at various stages together with the corresponding intake of that constituent by the plant. This could not be done as it was found that even when a soluble silicate was added to the soil it was very soon converted into insoluble forms consequent on certain interactions between the soil and the silicate (Sreenivasan, 1935). It would follow therefore that although at any one time there may not be a large quantity of soluble silica in the soil system, it does not give any indication of what might have been absorbed by the plant. In other words, the availability of silica to the plant is not measurable merely by the amount present in solution at any time.

The presence of such large quantities of silica in the rice plant under conditions of optimum growth might lead one to suppose that the supply of soluble silica is one of the most important factors governing the yield of crop. Observations have, however, been made by a number of workers in regard to the proportions of the various elements contained in plants of different species grown under the same condition and of the same species grown under different conditions and it is found that they depend upon a number of factors such as the species of plant, the type of soil, the distribution of the root system, the rainfall and general climatic conditions as well as the methods of cultivation. While therefore the amount of nutrients necessary for the optimum growth of plants at the different stages of their growth is an important factor from the standpoint of crop production, an analysis of the plant does not necessarily give any indication of the deficiencies or fertilizer needs of the soil (Salter and Ames, 1928).

The present enquiry, while providing data regarding the intake of silicon by the rice plant, does not entirely explain the significance of that element in the physiology of the plant. The fact that after 102 days there is not any further appreciable assimilation of silicon would mean that if at all silicon is an important factor in improving plant growth, it must perform its function before this period. But as to whether the increased assimilation of silicon precedes and is therefore the main factor governing the yield of paddy or whether it is only a secondary thing, can be definitely said only when observations on growth are correlated with corresponding intake of silicon at very frequent intervals of time during the life of the plant. Experiments are also necessary to show whether under conditions of dry cultivation and in the presence of free supply of soluble silica secured probably by addition of sodium silicate to the soil, the yield of paddy can be improved to the same level as when grown under swamp conditions. Trials have also to be conducted by growing paddy under a variety of conditions and at different seasons to be able to say whether or not silicon plays any important rôle in its nutrition. It is also necessary in order to know whether

silicon is a "limiting factor" in paddy nutrition to carry out experiments by growing rice to maturity and harvesting stage in solution cultures with and without the addition of silica in soluble form.

There may be certain other factors which are more important from the point of the rice plant for maintenance of swamp soil conditions and this latter condition induce a greater intake of silica enabling the plant to withstand such an environment which might ordinarily be detrimental to the crop. Thus, it is well known that the rice plant assimilates nitrogen only in the form of ammonia especially in the early stages of its growth, and under swamp conditions the transformations of added nitrogen stop at ammonia stage, practically no nitrate being formed. Thus swamping might be beneficial to the crop only inasmuch as it enables intake of nitrogen as ammonia; absorption of silica then might be taking place just because it is also formed in the soil under such conditions in fairly large quantities.

Since the extent of root system of the plant was observed to vary with different treatments being most in the swamped, manured series, it is possible that greater intake of minerals including silicon in these series, may be due to this factor alone without the latter being of any direct importance in the nutrition of the plant.

As to how exactly under swamped conditions more of silicon and other minerals are brought into solution, it is still obscure; there is little doubt however that the organic acids which are formed under such conditions play some part in the dissolution of minerals.

The mode of intake of silicon by the plant is still obscure. In fact, little is known regarding the manner in which plants assimilate their nutritive elements. Since different plants are composed of different proportions of the various elements, it has been assumed by some that they exercise a "selective absorption" and that the variations in composition are due to differences in the specific absorbing powers of the roots. Others consider that this is due to a specificity of the protoplasm, which requires different proportions of materials for its development in different parts. Thus, Dastur and Malkani (1933) state that the preferential absorption of ammonium ion by the rice plant at early stages of its growth and of nitrate ions at later stages is due to differences in the permeability of the protoplasm of the plant at the different periods and in a later communication (1934), Dastur and Kalyani correlate this behaviour with the *iso*-electric point of the proteins of the protoplasm of the tissues of the rice plant. Yet another school of thought (*cf.* Davis, Hoagland and Lipman) emphasise that the difference in the extents of the root systems and the differences in the rate of production

of carbon dioxide by these roots are factors which may determine, to a considerable degree, the differences in the amount of minerals absorbed by plants. It is as yet difficult to say whether the absorption of the elements of nutrition by the plants is a selective process in the strict sense of the term.

Another observation of interest that has emerged from this study is the sudden decrease or perhaps even cessation, towards the end, in the rate of absorption of the minerals by the plant. The fact that the percentage of silicon in the ash steadily increases with the age of the plant would naturally mean that the percentages of the other ash constituents decrease in the later stages of growth of the plant. Such a decreased rate of absorption of minerals during the ripening period has been reported by a few earlier workers in the case of other plants. Thus, Hornberger (1882) found in the case of corn that, when the tassels were beginning to form, there was an abrupt slowing down in the rate of absorption of the various elements. Similar observations have been made by Burd (1919) in the case of barley. It would therefore appear (Table XVI) that while the rate of absorption of elements by plants during the different stages of growth may vary considerably, in some plants, all the soil nutrients required for their further development are already accumulated in the stem at the time of flowering when their further absorption ceases almost completely.

Summary.

1. Periodical observations on rice plants grown under wet as well as dry soil conditions, with and without different organic manures, showed that (*a*) better growth and yield are obtained under swamp conditions than under "dry" ones, (*b*) there is a greater intake of minerals particularly silicon in the case of the swamped series over that in "dry" series, and (*c*) the percentage of silicon in plants grown in the swamped and manured soil is greater than in the others.

2. The significance of these observations in relation to (*a*) the possible beneficial effects of silicate fertilisation in field practice, and (*b*) the rôle of silicon in the nutrition of the rice plant have been discussed.

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ON TWO FORMS OF *HYDRURUS* AG. FROM KASHMIR.

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No member of the Chrysophyceæ,—a freshwater class of algæ characterised by the presence of brown or orange-coloured chromatophores and in great part naked protoplasts, and by the absence of starch,—has yet been recorded from India. The writer, on his way to Ladak (Kashmir) in June, 1931, collected two forms of *Hydrurus* Ag., described below. These two algæ were found attached to small stones, about 50 feet apart, submerged in shallow water of a narrow stream formed by melting of snow, at a distance of about three miles from Sonamarg, and at an altitude of about 9,000 feet above sea-level.

Hydrurus fœtidus (Vill.) Kirchn. Agardh, *Systema Algarum*, p. 24, 1824; Wolle, *Freshw. Alg. U.S.*, Pl. 164, 1887; Lagerheim, *Ber. Deutsch. Bot. Ges.*, VI, p. 73, 1888; Pascher, *Süssw.-flora Deutsch. Oster. u. d. Schw.*, 2, Flagellata II, Fig. 139, a-c, 1913; Fritsch, *Struct. and Reprod. Algæ* I, p. 547, Fig. 181, 1935.

Forma, (Fig. 1, *A*).

Length of threads, up to 40 cm.; diameter of spherical cells, 6-12 μ; length of ellipsoidal cells, up to 20 μ.

The length of the threads in this form is greater than that recorded for the type.

Habitat—Attached to submerged stones in a fast-flowing shallow stream, Sonamarg, Kashmir.

var. *indica* var. nov. (Figs. 1, *B*, and 2, *A-C*).

Thallus a branched system of thick reddish-brown and easily-breakable threads, composed of a soft, gelatinous and cylindrical axis, bearing long and short branches, distributed irregularly over its whole surface; both the main axis and the laterals being gradually attenuated towards their apices. Short branches of limited growth numerous, hardly seen with the naked eye, with their ends directed towards the apex of the main axis, simple when young (Fig. 2, *A*), richly branched when mature (Fig. 2, *B*) owing to the production of numerous short conical processes, arising usually irregularly and only occasionally in an acropetal order. Long branches of unlimited growth

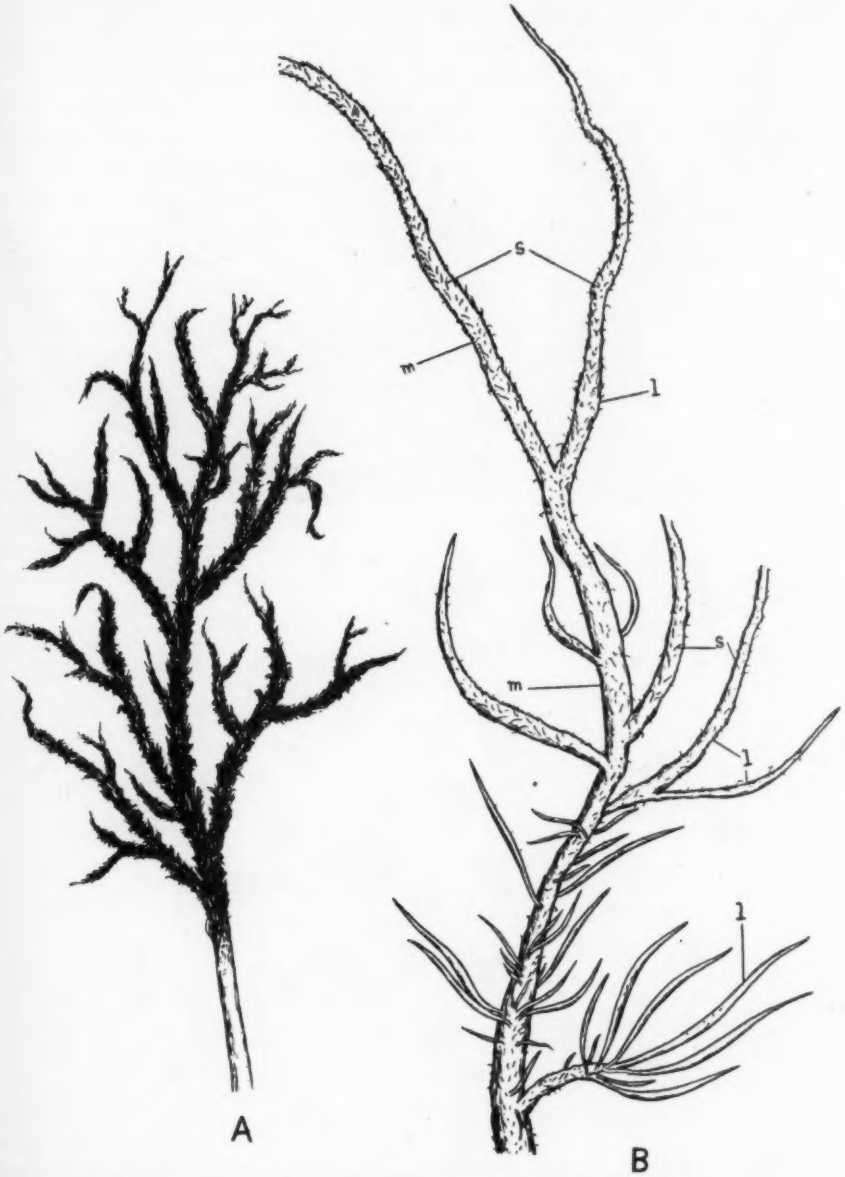

FIG. 1. *A—Hydrurus fœtidus* (Vill.) Kirchn. *forma*, and *B*—var. *indica* var. nov. showing habit. *l*, long lateral; *m*, main axis; *s*, short lateral. *A* × ⅔; *B* × 2·6.

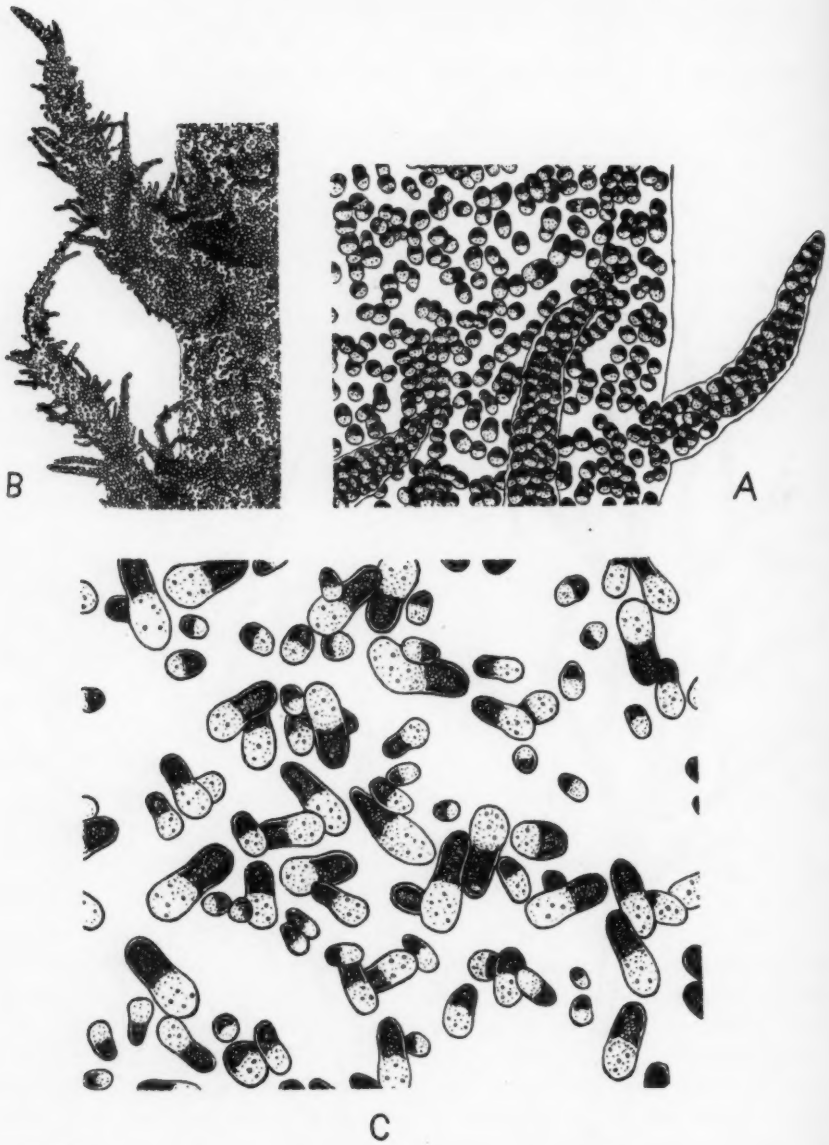

FIG. 2.—*Hydrurus foetidus* (Vill.) Kirchn. var. *indica* var. nov. *A* and *B*, portions of axis showing young and mature short laterals respectively; *C*, part of axis showing both young and mature cells of different forms. *A*×400; *B*×60; *C*×800.

sparingly formed, especially towards the apex of the main axis, and far less in number than the short ones, slightly attenuated towards the base when young, repeating the structure of the main axis, though generally not producing branches of unlimited growth. Cells naked, loosely aggregated in the centre than at the periphery in both the main axis and the laterals, at first almost spherical, later ellipsoidal, more or less dum-bell-shaped, club shaped or slipper-shaped (Fig. 2, *C*), with the posterior half, containing a few contractile vacuoles and some granules, usually broader than the anterior one, possessing a single chromatophore. Chromatophores cup-shaped in spherical cells, tumbler-shaped in elongated ones, without pyrenoids.

Length of threads, up to 10 cm.; breadth of threads at their bases, 3 mm.; diameter of spherical cells, 5-10 μ, rarely up to 12 μ; length of elongated cells, 24-40 μ, rarely up to 48 μ.

Habitat.—The same as for the above form.

This variety differs from the type on account of its reddish-brown colour in fresh condition, the axis being branched (though sparingly) throughout its length, and the plant-body being not constructed to form any feathery tufts with a plumose appearance. It further differs in its branches arising irregularly and not in an acropetal order, and in the mature cells being much more elongated.

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INVESTIGATIONS ON THE RÔLE OF SILICON IN PLANT NUTRITION.

Part III. On the Nature of Interaction of Soil or Hydrogels of Iron Oxide or Alumina with Mixtures of Phosphates and Silicates.

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Ever since the recognition that the colloidal fraction of the soil has the inherent property of converting soluble phosphates or phosphatic fertilisers into insoluble forms, various investigators (*vide*, Sreenivasan, 1935[1]) have attempted to clarify the behaviour of phosphates in the soil, but opinion is yet largely divided in regard to the exact mechanism of phosphate fixation. The principal functional fraction of the soil is made up of inter-related heterogeneous colloidal systems, the "ampholytes" (Mattson and Pugh, 1934) and as such phosphate retention by soils is complicated by a number of factors. Studies on phosphate adsorption with simpler systems of colloids have, in recent years, been carried out by a number of workers (Gordon *et al*, 1922, 1923; Miller, 1928; Ghosh and Bhattacharya, 1930; Mattson, 1927, 1930, etc.). More recently Demolon (1933), Demolon and Bastisse (1933, 1934), Ravikovitch (1934), Pugh (1934) and Bradfield *et al* (1935) have published papers dealing with the ionic exchange of phosphates.

In view of the earlier observations (Sreenivasan, *loc. cit.*) that soluble silicates interact with the soil in a manner similar to soluble phosphates, it was considered desirable to study the behaviour of solutions of phosphate and silicate together, when added to soil or pure gels of alumina or iron hydroxide. It was hoped that such a study may throw light on the mode of action of colloidal forms of silica in increasing phosphorus availability to cereal crops—an observation made by a number of independent workers, but on which highly conflicting views have been held, even upto recent times (Sreenivasan, 1934; Akhromeiko, 1934; and Dix and Rauterberg, 1934).

Experimental.

Materials: *Soil.*—This was a red loam from an adjoining uncultivated tract of land. The soil was air dried and ground to pass the 40-mesh sieve.

Silicate.—Kahlbaum's 25 per cent. sodium silicate solution was filtered free of suspended impurities and used as such. Each c.c. of this solution was equivalent to 0·25 g. anhydrous silica. *Phosphate.*—A standard solution of pure potassium dihydrogen phosphate (KH_2PO_4, A.R.) was employed in place of the soluble phosphate. 1 c.c. of this solution corresponded to 1 mg. of P_2O_5.

The P_H of the soil, the silicate (aq). and the phosphate (aq.) were 7·2, 10·6 and 4·6 respectively, as measured with the Hellige Comparator.

Method.—The quantities of silicon and phosphorus were estimated on the same samples, a special modified procedure being adopted for the purpose. The method may be outlined as follows :—The solution is evaporated to a small bulk on the water-bath and after cooling, spotted, with about 5 c.c. of pure concentrated sulphuric acid. It is again heated for a time (5 to 10 mins.) and then treated with about 10 c.c. of 95 per cent. alcohol. The mixture is allowed to remain on the water-bath for about 15 mins. after which it is diluted and filtered free of silica, the precipitate being washed well. To the filtrate and washings (about 100 c.c. in volume) is added enough solid ammonium nitrate to make it a 15–20 per cent. solution. It is then heated nearly to boiling and treated with 20 c.c. of a 10 per cent. solution of ammonium molybdate. The mixture is vigorously shaken and maintained near to boiling temperature for about 10 mins. The yellow precipitate is then filtered off, washed with cold water until free from acid and dissolved in standard alkali, excess of which is back titrated against acid with phenolphthalein as indicator. 1 c.c. normal alkali corresponds to 2·538 mg. P_2O_5 (*Cf.* Neumann, 1902, 1904).

Retention of Phosphate by the Soil.—With a view to determining the quantities of phosphate retained by the soil used in the present study, samples (100 g.) of the latter were treated with varying quantities of the phosphate solution and the mixtures allowed to stand for 30 mins. Enough water was then added to make the volume of suspensions to 200 c.c. in each case. They were then shaken in an end-over-end shaker for a period of 2 hours (trials having shown that further shaking was unnecessary), filtered and the phosphate present in aliquots of the filtrate determined (Table I).

Considerable quantities of the added phosphate are retained by the soil (converted into insoluble forms). With increasing amounts of added phosphate, there is also an increase in the quantities retained. But there is a gradual decrease in the corresponding percentage retention. The observations are thus similar to the retention of soluble silicates by the soil (Sreenivasan, *loc. cit.*).

TABLE I.

Phosphate added (as p.p.m. of P_2O_5)	0	20	50	100	150	200
Phosphate recovered (as p.p.m. of P_2O_5)	Nil	5·22	17·95	45·92	71·39	103·5
Per cent. of Phosphate retained by soil	..	73·9	64·1	54·1	52·4	48·2
P_H of extract	7·2	7·2	7·2	7·0	6·8	6·4

A study of the changes in the H-ion concentration of the soil extract shows that with small concentrations of phosphate, the soil reaction is practically unaffected (due to the buffering effect of the soil constituents) while, with increasing amounts, there is a steady shift in the P_H towards the acid side.

Retention of Phosphate by the Soil in Presence of Sodium Silicate.—In the following experiments, the soil (100 g. lots) was first treated with 5 c.c. of sodium silicate (corresponding to 1·25 g. SiO_2) and allowed to stand for half an hour. Known amounts of phosphate solution were then added and the suspensions let stand for another half an hour after which they were extracted as before with water. The phosphate in solution was then determined in each case. (Table II.)

TABLE II.

Phosphate added (as p.p.m. of P_2O_5)	0	20	50	100	150	200
Phosphate extracted (as p.p.m. of P_2O_5)	..	7·1	20·9	53·0	78·5	111·7
Per cent. retention of Phosphate	..	64·5	58·2	47·0	47·7	44·2
P_H of extract	8·0	8·0	7·8	7·8	7·4	7·2

From a comparison of the amounts of phosphate recovered with the corresponding figures in Table I, it will be seen that in the presence of the silicate greater quantities of phosphate are extracted from the soil. The percentage retention of phosphate in soils treated with sodium silicate is also less in the latter cases, the differences being more with small amounts of added phosphate. The H-ion concentration of the extract in the case of specimens

treated with silicate and phosphate are also less than in those treated with phosphate alone.

Influence of Reaction on the Retention of Phosphate by the Soil.—In the above experiments (Table II), the addition of sodium silicate to the soil has made the soil reaction somewhat alkaline and hence the extent of retention of phosphate by the soil with and without added silicate may not be strictly comparable. With a view to finding out the possible influence of the P_H of the soil or of the phosphate solution on phosphorus extractability, the following experiments were carried out in which the soil (100 g.) was adjusted to different initial P_H values by addition of varying amounts of normal hydrochloric acid or sodium hydroxide as the case may be, before treatment with a known amount (10 c.c.) of the phosphate solution. The recovery of phosphate in each case is given below (Table III).

TABLE III.

P_H (adjusted) of soil	3·0	4·6	6·4	7·2	9·0	9·8
Phosphate extracted (as p.p.m. of P_2O_5)	43·0	43·4	44·7	46·3	48·8	50·1

Phosphate originally added (as P_2O_5) = 100·0 p. p. m.

In another set of experiments, the phosphate solution (10 c.c.) was adjusted to different initial P_H values by addition of acid or alkali (decinormal), as the case may be, and then added to 100 g. lots of the soil. The suspensions were then extracted in the usual way. (Table IV.)

TABLE IV.

P_H (adjusted) of Phosphate solution	3·0	5·0	7·0	9·0	10·6
Phosphate extracted (as p.p.m. of P_2O_5)	43·8	46·0	47·3	48·5	49·4

Phosphate added (as p. p. m. of P_2O_5) = 100·0.

It may be observed that in either case (Tables III and IV) there is an increase in the amount of phosphorus recovered as the H-ion concentration decreases (*Cf.* Mattson, 1927, 1931 ; Gordon and co-workers, *loc. cit.*).

In view of the fact that with increased P_H there is also an increased recovery of added phosphate, it appeared probable that the influence of sodium silicate in increasing the amount of phosphorus in solution (Table II) may, in part at least, be due to the increased alkalinity brought about by

addition of silicate (Sreenivasan, *loc. cit.*). It was therefore thought desirable to overcome this influence of the sodium silicate on the soil reaction before the addition of phosphate. Accordingly, in the following experiments, 100 g. lots of the soil were mixed with 5 c.c. of the silicate solution. At the end of half an hour enough hydrochloric acid was added to neutralise the sodium silicate present and the soil suspension thoroughly mixed. (The quantity of acid necessary was determined by separate titration of the silicate solution against 1 per cent. HCl using phenolphthalein as indicator and found to be 1·6 c.c.). Known amounts of phosphate solution were then added to the soil suspension thus obtained and the quantities of phosphorus retained were then determined in the usual way. The results are given in Table V.

TABLE V.

Phosphate added (as (p.p.m. of P_2O_5)	20	50	100	150	200
Phosphate recovered (as p.p.m. of P_2O_5)	5·4	19·3	50·5	78·6	110·6
Retention of Phosphate per cent.	73·0	61·4	49·5	47·6	44·7

It may be noted that the recovery of added phosphate is distinctly less than when the soil-silicate mixture was not neutralised before addition of phosphate (Table II). A comparison of the figures with the corresponding values obtained when the soil was treated with the phosphate alone (Table I) would, however, show that the quantities of phosphorus present in solution continue to be appreciably more in the presence of the silicate. In other words, the addition of the silicate to the soil results in an increase in the recovery of added phosphate due in part to the increased alkalinity and in part also to the silicate itself.

Effect of Addition of Increasing Quantities of Silicate (aq.) on Phosphorus Recovery.—To 100 g. lots of the soil were added varying amounts of sodium silicate. At the end of half an hour, the soil-silicate suspensions were divided into two halves and in one set of samples, they were thoroughly mixed with such quantities of 1 per cent. HCl as would be just enough to neutralise the sodium silicate present. All the samples were then treated with known amounts (10 c.c. and 20 c.c. respectively) of the phosphate solution and the amounts of extractable phosphate determined in each case. Table VI gives the results obtained :—

TABLE VI.

Sodium silicate added as mg. SiO_2	Phosphate originally added (as p.p.m. of P_2O_5) = 100		Phosphate originally added (as p.p.m. of P_2O_5) = 200	
	Phosphate recovered as p.p.m. of P_2O_5			
	Without neutralisation of added silicate	After neutralisation of added silicate	Without neutralisation of added silicate	After neutralisation of added silicate
500	48·0	47·6	104·3	104·3
1250	53·0	50·5	111·7	110·6
2500	56·1	53·4	118·8	115·6
3750	58·7	55·1	123·6	119·0

It was found that in the experiments where the sodium silicate added was not neutralised with acid before treatment with phosphate, the extracts were highly coloured due to the soil organic matter having been brought into solution, especially where large amounts of silicate were employed. In such cases, the extracts were boiled with hydrogen peroxide to oxidise away the organic matter before removal of silica by treatment with sulphuric acid.

The results (Table VI) show that addition of increasing quantities of silica results in corresponding increase in the amounts of soluble phosphorus. The quantities present in solution are greater in the experiments where the silicate added was not neutralised. This is indeed to be expected, in view of the fact that the alkalinity of the silicate will decrease phosphate retention.

When the quantities of phosphorus in solution are plotted against the amounts of silicate added, a logarithmic curve (Fig. 1) is obtained.

These observations would suggest that the nature of interaction between the soil and a mixture of silicate (aq.) and phosphate is governed by the relative concentrations of the latter. It is possible that in presence of the silicate the soil adsorbing complex combines preferentially with the colloidal silica released as a result of addition of silicate so that the extent of phosphate adsorption decreases. As the amounts of added silicate increase, there is a corresponding decrease in phosphate adsorption.

Effect of Addition of Sodium Silicate (aq.) to Soil treated with Phosphate (aq.).—In the foregoing experiments the sodium silicate was added prior to treatment of the soil with phosphate. With a view to seeing whether

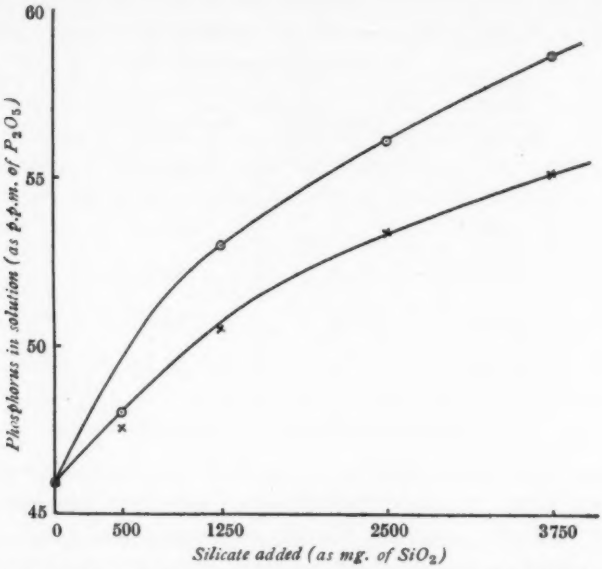

FIG. 1. Effect of Sodium Silicate (aq.) on Phosphorus Resorption.

⊙—⊙ without neutralisation of added silicate
×—× after " " "

addition of silicate after treatment of the soil with phosphate brings about any change in the extent of recovery of the added phosphate, some experiments were carried out in which the soil (100 g.) was first treated with a known amount (10 c.c.) of phosphate solution. At the end of half an hour, varying quantities of silicate were added to the mixture, followed in one set of experiments, by enough hydrochloric acid (1 per cent.) to neutralise the sodium silicate. In either case, the phosphorus in solution was determined in the usual way after extraction with water (Table VII).

There does not appear to be any difference in the amounts of soluble phosphorus whether the silicate is added after or prior to treatment with phosphate. In other words, phosphate adsorbed by soil is released from combination by silicate addition, presumably as a result of preferential adsorption of silica resulting from the latter.

Experiments with Silica Gel.—It has been shown that the addition of sodium silicate to soil is in effect the same as treatment with colloidal silica (Sreenivasan, *loc. cit.*). It was further observed that the behaviour of phosphates and that of soluble silicates towards the soil are similar in character but whereas in the former case, it is adsorption of an anion, in the latter it is

TABLE VII.

Sodium silicate added as mg. SiO_2	Phosphate extracted as p.p.m. of P_2O_5	
	Without neutralisation of added silicate	After neutralisation of added silicate
500	..	48·0
1250	52·2	50·9
2500	56·4	52·6
3750	..	56·4

Phosphate originally added (as p.p.m. of P_2O_5) = 100

adsorption of a negative colloidal complex. The behaviour of phosphate and silicate together towards the soil would appear to lend support to this view. With a view to observing whether the retention of phosphate by soil is affected in presence of colloidal silica as well, some experiments were carried out mixing soil (100 g.) with 10 g. of a silica gel (equivalent to 0·754 g. of SiO_2) prepared by careful neutralisation of a dilute solution of sodium silicate with hydrochloric acid and dialysis of the mixture until free from chloride. Known amounts of phosphate solution were then added to the mixture and the phosphorus extracted was determined in each case as before (Table VIII).

TABLE VIII.

Phosphate added (as p.p.m. of P_2O_5) ..	50	100	150	200
Phosphate extracted (as p.p.m. of P_2O_5)	18·4	48·8	74·7	107·3
Per cent. retention	63·2	51·2	50·2	46·3
P_H of extract	7·2	7·0	6·6	6·4

As distinct from the extracts obtained in the previous experiments with sodium silicate, it was observed that only very little of silica was extracted in the solution. A comparison of the results (Table VIII) with those in Table I would show that silica gel also increases the amount of water soluble phosphorus. Gile and Smith (1925) had observed that addition of 30 g. of silica gel increases somewhat the quantity of phosphoric acid brought into solution from rock phosphate by a mixed salt solution. Similar results

were obtained by Mattson (1922) on digesting a precipitated tricalcium phosphate with water suspensions of peat, ceramic clay and quartz flour.

Effect of Addition of Increasing Quantities of Silica Gel on Phosphate Retention.—100 g. lots of soil were thoroughly mixed with varying quantities of silica gel and the mixtures treated with a known amount (10 c.c.) of phosphate solution. The retention of phosphorus by the soil was then determined in the usual way. Results are given in Table IX.

TABLE IX.

Silica gel added (as mg. SiO_2)	377	754	1131	1508
Phosphate extracted (as p.p.m. of P_2O_5) ..	47·2	48·8	50·5	51·8

Phosphate added (as p.p.m. of P_2O_5) = 100.

It may be noted that as with sodium silicate, increasing quantities of silica gel increase the resorption of phosphorus.

A comparison of the figures for phosphorus in solution in the experiments with silica gel (Tables VIII and IX) with the corresponding values in the experiments with sodium silicate (Table VI) would show that the efficiency of phosphate resorption by the gel is somewhat less than that of the silicate solution. As shown in a subsequent section, silica gel itself has the power of adsorbing phosphate from solution although there is only slight adsorption. This adsorbed phosphate is, however, washed free from the adsorbing gel by the use of hot water. Since the extracts obtained in the above experiments with soil and silica gel were only cold water extracts, this adsorption of phosphate by silica gel might explain to some extent the lower values for phosphorus in solution obtained in these cases. Another explanation may be that the silica gel may not be as adsorptively active as the colloidal silica formed in the soil as a result of addition of sodium silicate. It is not unlikely that in the latter case the silica is formed in the *sol* condition and is hence more potent in regard to the release of phosphorus.

Absorption of Silica and of Phosphates in soluble forms by Colloidal Oxides of Iron and Aluminium.

Since the seat of interaction between the soil and silicate or phosphate is in the colloidal fraction, it was thought desirable to study the behaviour of mixtures of silicate and phosphate towards pure hydrous gels of iron oxide and alumina. The gels for the following studies were prepared as outlined previously (Sreenivasan, 1935[2]).

Adsorption of Phosphate by Silica Gel.—5 g. lots of hydrous silica gel (377 mg. SiO_2) were treated in stoppered bottles with 100 c.c. of a solution containing different known amounts of phosphate. The mixture was, in each case, shaken repeatedly and let stand overnight. They were then filtered and the phosphorus in aliquots of the filtrate estimated as outlined before. The total phosphorus in solution was calculated, the water present in the gels being also taken into account so that the retention was expressed on the dry weight of the gel. The results are recorded in Table X.

TABLE X.

Phosphate added (as p.p.m. of P_2O_5)	Phosphate in solution after adsorption (as p.p.m. of P_2O_5)	Phosphate retained by gel (as p.p.m. of P_2O_5)	Per cent. retention of P_2O_5	Retention of P_2O_5 per g. dry gel (p.p.m.)
20	18·4	0·6	3·0	1·6
50	48·0	2·0	4·0	5·3
100	96·4	3·6	3·6	9·6
200	193·5	6·5	3·3	17·3

It may be observed that only small quantities of phosphate are retained by the silica gel (*cf.* Gordon *et al, loc. cit.*). As is to be expected, there is an increase in the quantity of phosphate retained, with increasing concentration of phosphate, but there is a decrease in the percentage retention.

With a view to seeing whether the adsorbed phosphate is firmly held by the silica gel and is therefore not leachable with hot water, the gel-silica complex in the last case (wherein 200 p.p.m. of phosphate as P_2O_5 had been added to 5 g. of hydrous SiO_2 gel) was treated with successive 100 c.c. portions of hot water and the phosphorus in the filtrates determined separately. The results are given in Table XI.

It would be seen from the above that even the small quantity of phosphate retained is practically completely leached out in the first one or two washings. This was further confirmed by taking a portion of the washed gel on the filter, decomposing it with dilute sulphuric acid and subsequently testing qualitatively for phosphate when a negative test was obtained.

Effect of Addition of Different Quantities of Phosphate to Iron and Alumina gels.—5 c.c. of each of the gels of iron oxide and alumina (containing respectively 164 and 116 mg. of Fe_2O_3 and Al_2O_3) were treated in stoppered bottles with 100 c.c. of a solution containing known amounts of phosphate.

TABLE XI.

Phosphate added as P_2O_5	= 200 p.p.m.
Phosphate recovered from solution (as P_2O_5)	= 193·5 p.p.m.

	P_2O_5 (p.p.m.) in 100 c.c. wash water from gel-phosphate complex
First 100 c.c. ..	3·97
Second 100 c.c. ..	0·84
Third 100 c.c. ..	Nil

The mixtures were well shaken and allowed to attain equilibrium. (Usually they were left as such for 36-48 hrs. when steady values were obtained for the phosphate in solution.) They were then filtered and the phosphate in aliquots of the filtrate estimated. The total phosphorus in solution was calculated, the water in the gels being also taken into account so that minimum adsorption might be shown. The H-ion concentration of the phosphate solution before and after adsorption was also determined in each case colorimetrically. The results are given in Table XII.

TABLE XII.

Phosphate added (as p.p.m. of P_2O_5)	Initial P_H of phosphate solution	Iron hydroxide = 164·0 mg. Fe_2O_3. P_H = 7·6				Alumina = 116·0 mg. Al_2O_3. P_H = 8·0			
		Phosphate in solution after adsorption (as p.p.m. of P_2O_5)	Retention of phosphate per cent.	P_2O_5 retained per g. dry gel (p.p.m.)	P_H of phosphate solution after adsorption	Phosphate in solution after adsorption (as p.p.m. of P_2O_5)	Retention of phosphate per cent.	P_2O_5 retained per g. dry gel (p.p.m.)	P_H of phosphate solution after adsorption
20	7·0	6·7	66·5	81·1	7·2	6·3	68·5	118·1	7·6
50	6·8	16·7	66·6	203·1	7·0	16·3	67·4	290·5	7·6
100	6·8	33·4	66·6	406·1	7·0	38·4	61·6	531·0	7·4
150	6·8	54·7	63·6	581·2	7·0	64·3	57·2	738·7	7·2
200	6·6	82·2	58·9	718·5	7·0	100·2	49·9	860·2	7·0
300	6·4	138·4	53·9	985·4	6·8	156·6	47·8	1236·0	..

It may be seen from Table XII that adsorption similar to that with the soil takes place with the gels also (*cf.* Table I). Adsorption increases with increasing amounts of added phosphate, while there is a decrease in the percentage adsorption as the concentration increases.

Effect of Addition of Sodium Silicate on Phosphate Adsorption by Gels.— The gels (5 c.c. each) of iron oxide and alumina were first mixed with 10 c.c. of sodium silicate solution and then treated with varying quantities (in 90 c.c.) of phosphate solution as before. The phosphate solutions in one set of experiments contained enough hydrochloric acid (3·2 c.c. of 1 per cent.) as would neutralise the silicate present. The amounts of soluble phosphorus in solution at the end of 48 hours, with and without neutralisation, are recorded in Table XIII.

TABLE XIII.

Phosphate added as (p.p.m. of P_2O_5)	Not neutralised for the alkalinity of sodium silicate				Neutralised for the alkalinity of sodium silicate		
	Phosphate in solution as p.p.m. of P_2O_5	Percentage retention of phosphate	P_2O_5 adsorbed per g. of dry gel (p.p.m.)	P_H of phosphate solution after absorption	P_2O_5 in solution (p.p.m.)	Per cent. retention of phosphate	P_2O_5 adsorbed per g. of dry gel (p.p.m.)
Ferric hydroxide gel = 164·0 mg. Fe_2O_3							
20	8·4	58·0	70·8	9·5	7·9	60·5	73·8
50	21·5	57·0	173·8	9·4	19·4	61·2	186·6
100	39·9	60·1	366·5	9·2	38·6	61·4	374·4
150	69·9	53·4	488·4	9·0	61·4	59·0	540·3
200	95·6	52·2	636·6	9·0	89·3	55·4	674·8
300	154·9	48·4	884·9	8·6	147·0	51·0	933·0
Alumina gel = 116·0 mg. Al_2O_3							
20	8·4	58·0	100·0	..	7·5	62·5	107·7
50	22·3	55·4	238·8	9·8	19·2	61·6	265·6
100	52·2	47·8	412·0	9·8	45·1	54·9	473·3
150	76·0	49·4	637·8	9·6	69·7	53·6	692·1
200	118·0	41·0	706·8	9·4	109·6	45·2	779·3
300	175·6	41·5	1073·0	9·0	..	..	..

It would be seen from a comparison of the figures for soluble phosphorus in Tables XII and XIII, that the addition of sodium silicate has resulted in greater amounts of phosphorus being brought into solution. In the experiments where the effect of the added silicate on the reaction of the medium had been neutralised by addition of the requisite amount of acid, the increase is less than in the series where no acid was employed to neutralise the sodium silicate. But the P_H of the extracts in the latter cases are also very much towards the alkaline side.

Order of Addition of Phosphate and Silicate.—In the following experiments known amounts of the gels of iron oxide and alumina were first treated with 90 c.c. of a solution containing definite known amounts of phosphate. At the end of 24 hours they were treated with 10 c.c. of the silicate solution, followed by enough hydrochloric acid to neutralise the latter. The mixture was well shaken and set aside for another 24 hours after which the amounts of phosphate in solution were estimated as before (Table XIV).

TABLE XIV.

P_2O_5 originally present in solution (p.p.m.)	Ferric hydroxide gel = 164·0 mg. Fe_2O_3			Alumina gel = 116·0 mg. Al_2O_3		
	P_2O_5 in solution after adsorption (p.p.m.)	Retention per cent. of phosphate	P_2O_5 adsorbed per g. dry gel (p.p.m.)	P_2O_5 in solution after adsorption (p.p.m.)	Retention per cent. of phosphate	P_2O_5 adsorbed per g. dry gel (p.p.m.)
50	19·6	60·8	185·4	18·8	62·4	268·9
100	38·8	61·2	373·3	44·9	55·1	475·0
150	60·5	59·6	545·8	70·6	53·0	684·4
200	90·8	54·6	665·9	111·9	44·1	759·5

It was observed that as with the soil, there was not any significant difference in the values for soluble phosphorus by reversing the order of addition of silicate and phosphate. Irrespective of the order of addition of the two reagents, the presence of silica in solution definitely tends to increase phosphorus solubility.

Effect of Addition of Increasing Quantities of Sodium Silicate.—The gels (5 c.c. each) of iron oxide and alumina were treated with a known amount of a solution of phosphate and allowed to stand overnight. Different known quantities of sodium silicate were then added followed by 1 per cent. hydrochloric acid, just enough to neutralise the silicate. After thorough shaking,

the mixtures were let stand until equilibrium was attained. The phosphorus in solution was then determined in each case. Results are given in Table XV.

TABLE XV.

Silicate added as mg. of SiO_2	Iron hydroxide gel = 164·0 mg. Fe_2O_3		Alumina gel = 116·0 Al_2O_3	
	P_2O_5 in solution after adsorption (p.p.m.)	P_2O_5 adsorbed per g. dry gel	P_2O_5 in solution after adsorption (p.p.m.)	P_2O_5 adsorbed per g. dry gel
0	33·4	406·1	38·4	531·0
500	34·9	397·0	40·1	516·3
1250	36·5	387·3	42·2	498·2
2500	38·8	373·3	44·9	475·0
3750	41·3	358·0	48·0	448·2
5000	44·7	337·2	50·1	430·1

Phosphate originally present in solution (as p.p.m. of P_2O_5) = 100.

Effect of Addition of Silica Gel on Adsorption of Phosphate by Gels of Iron oxide and Alumina.—In the following experiments 5 c.c. each of the gels of iron oxide and alumina were mixed with 20 g. of silica gel (hydrous) before treatment with varying known quantities of phosphate. The extent of phosphate adsorption was then determined in each case (Table XVI).

TABLE XVI.

Phosphate added (as p.p.m. of P_2O_5)	System: Iron hydroxide + Silica gel			System: Alumina + Silica gel		
	P_2O_5 in soln. after adsorption (p.p.m.)	Per cent. retention of phosphate	P_2O_5 retained per g. of Fe gel (p.p.m.)	P_2O_5 in soln. after adsorption (p.p.m.)	Per cent. retention of phosphate	P_2O_5 retained by g. of Al gel (p.p.m.)
50	17·6	64·8	197·5	18·4	63·2	272·4
100	36·4	63·6	387·9	41·6	58·4	503·4
200	86·8	56·6	690·4	103·4	48·3	832·8

(Silica gel added = 1504 mg. anhydrous SiO_2).

It may be seen that addition of silica gel also aids resorption of phosphate although as already observed the efficiency of silica gel is less than that of an equivalent quantity of sodium silicate.

Influence of H-ion Concentration on Phosphate Retention.—In the experiments on the effect of addition of sodium silicate on phosphate retention

(Table XIII), it was found that in the cases where the reaction mixture was not neutralised for the alkalinity of the silicate, higher values for the phosphorus in solution were obtained. With a view to knowing the influence of H-ion concentration on phosphorus recovery from gels of alumina and iron hydroxide under the conditions of the experiments, the following experiments were carried out in which the gels (5 c.c. each) were treated with 100 c.c. of a solution containing a known amount of phosphate, but adjusted to different initial P_H values by use of decinormal acid or alkali as the case may be. After standing for 48 hours, the mixtures were filtered and the phosphate in aliquots of the solution determined in each case as before. (Table XVII.)

TABLE XVII.

Initial P_H of phosphate soln.	Iron hydroxide gel = 164·0 mg. Fe_2O_3				Alumina gel = 116·0 mg. Al_2O_3			
	P_H of solution after adsorption	Phosphate in solution (p.p.m. of P_2O_5)	Phosphate retained per cent.	Phosphate retained per g. gel (p.p.m. of P_2O_5)	Phosphate in solution (p.p.m. of P_2O_5)	P_H of solution after adsorption	Phosphate retained per cent.	Phosphate retained per g. gel (p.p.m. of P_2O_5)
2·5	2·6	29·6	70·4	429·3	2·6	33·8	66·2	570·7
5·0	5·0	32·0	68·0	414·7	5·2	36·1	63·9	550·8
7·0	7·2	33·0	67·0	408·6	7·4	38·6	61·4	529·3
9·0	8·6	35·1	64·9	395·8	8·8	42·0	58·0	499·9
10·1	10·0	36·7	63·3	386·0	10·2	45·1	54·9	473·3

Phosphate added (as p.p.m. of P_2O_5)= 100·0.

As has been observed already by Mattson (*loc. cit.*) and by Gordon and co-workers (*loc. cit.*), it may be seen that with increase in the P_H there is a decrease in the retention of phosphate. The changes are similar in character to those observed on the addition of sodium silicate of varying initial P_H values to gels of iron oxide and alumina (Sreenivasan, *loc. cit.*).

In another set of experiments, the gels were treated with 100 c.c. of a solution containing a known amount (10 c.c.) of phosphate together with 10 c.c. of silicate solution, the mixture having been initially adjusted to varying P_H values. The extent of phosphate adsorption in the several cases as determined in the usual way is recorded in Table XVIII.

A comparison of the values for phosphate in solution at any particular P_H with and without sodium silicate would show that addition of that reagent decreases phosphate adsorption by the gels. However, the extent of phosphate resorption is greater at low P_H than at high ones. Since at high

TABLE XVIII.

Initial P_H of silicate phosphate mixture	Iron hydroxide gel = 164·0 mg. Fe_2O_3			Alumina gel = 116·0 mg. Al_2O_3		
	P_2O_5 in solution (p.p.m.)	P_2O_5 adsorbed (per cent.)	P_2O_5 adsorbed per g. dry gel (p.p.m.)	P_2O_5 in solution (p.p.m.)	P_2O_5 adsorbed (per cent.)	P_2O_5 adsorbed per g. dry gel (p.p.m.)
2·5	36·3	63·7	388·5	42·3	57·7	497·4
5·0	37·6	62·4	380·6	43·4	56·4	486·2
7·0	38·6	61·4	374·4	44·9	55·1	475·0
9·0	40·3	59·7	364·1	46·6	53·4	460·3
10·6	42·2	57·8	352·5	49·1	50·9	438·7

Phosphate originally present (as p.p.m. of P_2O_5) = 100. Silicate present (as mg. of SiO_2) = 2500.

hydrogen-ion concentrations, the adsorption of silicate is most (Sreenivasan, *loc. cit.*), it would appear that phosphate resorption is the result of silicate adsorption. In other words, conditions which favour adsorption of silicates by the colloidal adsorbing complex result in maximum efficiency of the silicate in regard to release of phosphates from combination.

Discussion.

It has been stated in the previous communications (Sreenivasan, *loc. cit.*) that the reaction of soil or of colloidal oxide of iron or aluminium with alkali silicate resolves itself into interaction with dilute alkali on the one hand, and colloidal silica on the other. Both these reactions would naturally tend to alter the extent of retention of soluble phosphate by the soil adsorbing complex or the gels of iron oxide or alumina as the case may be. Although it has been known (*vide*, Voelcker, 1915, 1916; Russell, 1932) that the application of small quantities of alkali is beneficial to crop growth, it may yet be observed that in regard to the mode of action of sodium silicate in increasing phosphorus resorption, the effects of the silica formed—presumably in the *sol* condition—is far more than the cationic effect.

During recent years, evidence has been adduced to show that phosphate adsorption by soil colloids is an anion exchange phenomenon (Mattson, *loc. cit.*; Ravikovitch, *loc. cit.*; Pugh, *loc. cit.*; Scarseth, 1935; and others). It would, however, appear from the present enquiry that the behaviour of the soil towards silicate or phosphate in presence of varying concentrations of H-ion is different from its behaviour towards mixtures of phosphate and silicate in solution. Thus, in the former case, the extent

of adsorption of phosphate or of silica by the adsorbing complex decreases with increasing P_H. This would suggest that the nature of the adsorbing complex or its ability to retain varying amounts of phosphates or silicates from solution is altered by the H-ion concentration. On the other hand when phosphate and silicate are present together in solution, it would appear that the adsorbing colloid preferentially combines with the silicate, thereby resulting in greater amounts of phosphates being present in solution. Even when the silicate is added to the soil after treatment with phosphate, the latter is released from combination. In other words, the soil-silicate complex is more stable than the soil-phosphate complex, and hence, addition of silicate solution to the latter results in resorption of phosphate. Whether or not the quantities of phosphates thus exchanged correspond to the amounts of silica adsorbed is not known.

Although the direct effects of soluble silica in increasing the water-soluble phosphorus may only be small, yet it is not improbable that under field conditions and in presence of the growing plant, the actual amounts of phosphorus made available to the plant will be quite significant. Indeed as is evident from a number of earlier researches (Schollenberger, 1922; Lemmermann, Wiessmann and Sammett, 1925; Brenchley, Maskell and Warington, 1927; and others), the increased availability of phosphorus to plants consequent on silicate fertilisation should be far more than what is indicated in the water extract. This would mean that silica, in some unknown manner, alters the nature of the unavailable phosphates in the soil whereby a part of the latter becomes available to the plant without its becoming water-soluble. For a fuller understanding of the precise mechanism by which silicate increases the intake of phosphorus by plants from the soil, it is necessary to have a reliable measure of the available phosphorus in soils.

The action of sodium silicate in increasing phosphorus resorption from soil would no doubt depend, on a number of factors, the most important among which are the nature of the soil and the relative proportions of the silicate and phosphate. Addition of sodium silicate to soil would tend to make the soil reaction alkaline, although when the quantities are small, the silicate may not seriously affect the physical properties of the soil, especially when the latter is rich in buffering constituents. It would be useful, however, to conduct large-scale field trials with silicate, either by itself, or in combination with other fertilisers, so as to determine the proportions which would give maximum beneficial effects, under all conditions, and with all types of soil without any injury to the soil itself.

Summary.

(1) When soil is treated with a solution of a phosphate a considerable part of the phosphate is retained by the soil. As the concentration of the phosphate is increased, the quantities of phosphate retained also increase, although there is a decrease in the percentage retention of phosphate.

(2) The extent of retention of phosphate by the soil decreases considerably when the soil is treated with a solution of sodium silicate prior to addition of phosphate. The reaction of the mixture however changes towards alkalinity.

(3) The extent of phosphate retention decreases with increase in P_H value of the medium. When soil is mixed with sodium silicate together with enough acid to neutralise the latter prior to treatment with phosphate solution, its power of retaining phosphate is somewhat more than when sodium silicate alone is added, but is still less than that of the soil alone.

(4) Addition of increasing quantities of silicate to soil prior to addition of phosphate decreases the extent of retention of the latter although the decrease is not in strict proportion to the quantities of silicate employed.

(5) Reversing the order of addition of silicate and phosphate to soil does not appreciably alter the extent of retention of phosphate by the soil.

(6) Addition of silica gel to soil results in similar decrease in phosphate retention although the effect is less marked than with corresponding quantities of sodium silicate.

(7) Silica gel has the power of adsorbing phosphate from solution only to a small extent and the adsorbed phosphate is practically completely leachable by hot water.

(8) The behaviour of sodium silicate and of phosphate in solution in presence of hydrogels of iron oxide and alumina is very similar to that with the soil. The effect of sodium silicate in decreasing phosphate retention by the colloid is likewise more pronounced when the silicate is not neutralised for its alkaline reaction.

(9) Study of the influence of hydrogen-ion-concentration of silicate-phosphate mixtures on phosphate retention by gels of iron oxide and alumina shows that there is a decrease in the extent of retention of phosphate by the gel in presence of silicate at all P_H values. The decrease is greater at low P_H values than at high ones.

(10) Based on the foregoing and other observations, the mechanism of the increased resorption of phosphorus consequent on application of silicate has been discussed. The nature of further work relating to (*a*) the standardisation of a suitable procedure for measuring the available

phosphates in soil, and (*b*) its application in the elucidation of the mode of action of colloidal silica in increasing phosphorus availability is discussed.

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INVESTIGATIONS ON THE RÔLE OF SILICON IN PLANT NUTRITION.

Part IV. Effect of Silicate Fertilisation on the Growth of the Rice Plant and Yield of Paddy.

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Although it has long been recognised that the rice plant requires maintenance of swamp soil conditions for optimum growth and yield of grain, yet very little definite information is available regarding the need for such excessive water requirement of the crop. It has been suggested (Nanji and Shaw, 1925) that the high silicon content of the different parts of the rice plant may in some way be due to the peculiar (swamp) soil conditions under which that plant is generally cultivated. Indeed, comparative studies on the intake of silicon under conditions of dry as well as wet cultivation (Sreenivasan, 1936) have shown that the plant absorbs considerably greater quantities from the soil system in the latter than in the former cases. The availability of silica (Sreenivasan, 1935) has also been found to increase with moisture content of the soil being greatest in flooded soils. Observations by Suzuki (1935) on the effect of variations in soil moisture on the susceptibility of the rice plant to helminthosporium and blast diseases have also shown that susceptibility increases with the aridity of the soil. The susceptibility seems to be closely connected with the thickness of the outer wall and the silicated outermost layer of the epidermal cell which was greatest in the flooded series. Rate of infection in arid and in flooded soils alike decreases with supply of soluble silica. Other workers (Miyake and Adachi, 1922; Kawashima, 1927; Miyake and Ikeda, 1932, etc.) have also reported that the resistance of the plant to disease is closely connected with the silica content of the plant, the latter increasing with the quantity of silicates applied to the soil.

The foregoing observations bring out the importance of silicon in the nutrition of the rice plant. They do not, however, entirely explain its significance in the physiology of the crop. The presence of such large quantities of silica in the plant under conditions of optimum growth (Sreenivasan, *loc. cit.*) would suggest that the supply of soluble silica may be an important

factor governing the growth of the plant and yield of grain. The present experiments which are only preliminary in character were designed to show whether under conditions of dry cultivation and in presence of a free supply of soluble silica secured by the addition of sodium silicate to the soil, the yield of paddy can be improved to the same level as when grown under swamp conditions.

Experimental.

Glazed earthenware pots were made up in the usual way with 50 lbs. each of a clay soil (3 mm. sieve) from an adjoining area. The pots were then divided into four series (each of 50) as follows :—(*a*) unmanured and maintained at 60 per cent. saturation ; (*b*) unmanured and puddled with water ; (*c*) manured with 100 g. of green manure per pot and maintained as in (*a*) ; (*d*) manured as in (*c*) but maintained as in (*b*).

The green manure was applied in the form of fresh leaves of *Pongamia glabra* cut into small bits and was thoroughly incorporated into the soil to a depth of about 6″.

The pots in each series were again sub-divided into five groups as follows :—(1) untreated (control) ; (2) treated with 2 g. per pot of potassium phosphate applied as a 10 per cent. solution ; (3) treated with 20 c.c. of a 25 per cent. solution of Kahlbaum's sodium silicate applied after diluting five times ; (4) treated with 80 c.c. of 25 per cent. sodium silicate applied also after being diluted five times ; and (5) treated as in (4) but the silicate was added in four equal instalments during the life of the crop.

Phosphate was employed in one of the treatments (Group II) in view of the fact that the application of the silicates is known to increase phosphorus availability to the plant (Sreenivasan, 1934). The soil used in the present set of experiments was known to be deficient in phosphorus and hence any effect that the added silicate may have in stimulating plant growth by increased intake of phosphorus will be only insignificant. However, it was thought desirable to run a check series with phosphate in order to overcome any effect the added silicate may have in this direction.

There were thus 20 treatments in all with 10 pots for each treatment.

The pots in series (*b*) and (*d*) were made as above on 22nd May 1935, while those in series (*a*) and (*c*) were made on 31st May 1935. The chemicals were all added on 4th June 1935 and where the sodium silicate was added in instalments (Group V), the first addition was made on the above date while the subsequent additions were made as above on 25th June 1935, 15th July 1935, and 15th August 1935 respectively.

The above dates corresponded roughly with the stages of tillering, active vegetative growth and flowering respectively. In view of the observation that the intake of silicon by the plant ceases after the flowering stage (Sreenivasan, 1936) all the silicate was added before this period in the life of the crop.

Paddy (variety Adt. 3, a short duration *Kar* crop) was sown in separate seed beds on 8th May 1935. The seedlings were transplanted into the experimental pots on 6th June 1935 when they had attained an average height of about 6 inches. Six seedlings were planted to each pot. When the seedlings had become well established in the soil, enough water was added to the pots in series (*b*) and (*d*) so as to keep the soil well under submergence (3 inches). Slow drainage was allowed, water being added at frequent intervals to keep the level of submergence nearly constant. This was continued until about the middle of September when grains had formed in all the cases. Pots in series (*a*) and (*c*) were just kept moist by daily addition of water, which was also stopped during the ripening stage.

Results.

The growing period was from 6th June 1935 to 6th October 1935. Systematic periodical records of the maximum height of the plants in each pot and the number of tillers of every plant were maintained until the harvesting date. The different developmental stages of the plant together with the approximate dates are given below.

Germination on 14th May 1935.
Transplantation on 6th June 1935.
Tillering stage (about) 20th July 1935.
Flowering stage (about) 2nd September 1935.
Panicle formation (about) 15th September 1935.
Harvesting on 6th October 1935.

The average number of tillers per plant as also the maximum height of the above ground portion of the plant in each group under the different series at different times are given in Table I.

The yield of grain and of straw on harvesting are recorded in Table II. The results represent the total yield (in g.) of all the plants (60) in each series.

Discussion of Results.

Addition of phosphate increases tillering and also the number of earing tillers. The proportion of grain formed also increases considerably. Phosphate was also found to have a quicker ripening effect (Hall and Morison, 1906). The effect of phosphate treatment was nearly the same in the manured as well as unmanured series, both arid and flooded.

TABLE I. *Tillering and height at various stages of growth.*
(*Figures represent the data per plant being the average of* 60 *plants in a series.*)

Treatment Group		Age of Plant—Days															
		Unmanured arid series				Unmanured flooded series				Green manured arid series				Green manured flooded series			
		67	111	124	145	67	111	124	145	67	111	124	145	67	111	124	145
(i) Untreated check.	Number* of tillers	2·0	3·4 (1·1)	3·8 (2·0)	1·4 (0·6)	2·2	3·7 (1·0)	4·0 (3·2)	3·6 (3·3)	3·0	4·6 (1·3)	5·2 (2·0)	4·6 (3·4)	4·5	8·4 (5·0)	8·8 (8·0)	9·0 (8·4)
	Maximum height (cms.)	40	50	64	55	43	60	67	58	45	66	69	61	54	80	94	86
(ii) Treated with phosphate.	Number* of tillers	2·8	3·6 (2·4)	3·2 (2·8)	1·8 (1·4)	2·7	4·0 (2·2)	4·4 (3·7)	4·0 (2·9)	3·4	5·0 (3·1)	5·6 (3·2)	4·7 (3·2)	6·0	9·2 (6·2)	10·8 (9·1)	9·9 (8·2)
	Maximum height (cms.)	43	57	61	50	47	62	70	54	48	65	72	57	57	85	99	85
(iii) Treated with silicate, small quantity.	Number* of tillers	2·2	3·5 (2·2)	3·0 (2·7)	1·6 (1·1)	2·0	3·6 (2·0)	4·4 (3·6)	3·8 (3·4)	3·0	4·4 (2·7)	5·4 (3·4)	4·7 (3·0)	5·6	10·0 (5·4)	10·4 (8·2)	9·6 (8·0)
	Maximum height (cms.)	36	48	62	54	40	58	57	60	42	63	70	62	52	76	92	80
(iv) Treated with silicate, large quantity.	Number* of tillers	..	3·5 (1·8)	3·4 (2·9)	2·2 (1·5)	..	3·9 (1·9)	4·6 (3·9)	4·1 (2·7)	3·0	4·6 (2·8)	6·0 (4·2)	4·7 (3·0)	4·2	9·8 (6·3)	11·0 (9·4)	9·8 (8·0)
	Maximum height (cms.)	38	46	69	58	40	60	69	59	43	63	71	64	49	77	98	90
(v) Treated with silicate, large quantity in instalments.	Number* of tillers	2·0	3·2 (0·8)	4·0 (2·6)	2·0 (1·0)	2·4	3·8 (2·0)	4·5 (3·9)	3·8 (2·6)	2·9	5·0 (2·8)	6·2 (3·7)	5·1 (3·4)	5·8	9·2 (5·4)	11·0 (9·5)	10·0 (8·0)
	Maximum height (cms.)	38	50	63	52	45	64	71	58	45	67	74	59	56	84	96	87

* Bracketed figures are the number of earing tillers per plant (average), while the first figure represents the total number (earing and non-earing) of tillers.

TABLE II.

Yield of grain and straw (total of 60 plants in each case).

Treatment		(*a*) Unmanured—arid			(*b*) Unmanured—flooded			(*c*) Green manured—arid			(*d*) Green manured—flooded		
		Yield of grain (gms.)	Yield of straw (gms.)	Grain/Straw ×100	Yield of grain (gms.)	Yield of straw (gms.)	Grain/Straw ×100	Yield of grain (gms.)	Yield of straw (gms.)	Grain/Straw ×100	Yield of grain (gms.)	Yield of straw (gms.)	Grain/Straw ×100
(1) Untreated check	Fresh wt.	19·2	138·6	..	30·6	211·2	..	80·4	340·2	..	286·8	1185·6	..
	100° dry wt.	14·4	44·4	32·4	22·8	66·0	34·5	60·0	114·6	52·4	218·4	394·8	55·3
(2) Treated with phosphate	Fresh wt.	48·0	175·2	..	91·2	252·0	..	186·6	666·4	..	373·8	1464·0	..
	100° dry wt.	38·4	60·0	64·0	67·2	85·2	78·9	138·0	226·8	60·8	278·4	486·0	57·3
(3) Treated with silicate =S	Fresh wt.	54·0	150·0	..	76·8	205·8	..	236·4	766·8	..	360·0	1173·6	..
	100° dry wt.	40·8	49·2	82·9	56·4	73·2	77·0	180·6	261·0	69·2	276·0	402·0	68·7
(4) Treated with silicate =4S	Fresh wt.	60·6	157·2	..	73·2	228·6	..	251·4	806·4	..	384·0	1296·0	..
	100° dry wt.	46·2	54·0	85·6	54·6	81·6	66·9	194·4	273·0	71·2	288·0	427·2	67·4
(5) Treated with silicate =4S in instalments	Fresh wt.	60·0	152·4	..	82·8	228·0	..	264·0	768·0	..	433·2	1261·8	..
	100° dry wt.	45·6	50·4	90·5	64·2	76·2	84·3	199·2	259·8	76·7	334·8	423·0	79·1

Addition of small amounts of silicate definitely increases growth as well as yield in all the cases. Addition of larger amounts of silicateinc reases growth and yield to a greater extent, but this is not in proportion to the amount of silicate added. It was noticed that in these cases, there was a general set-back on the growth of the plants in the early stages due no doubt to excessive alkalinity. The plants, however, tillered actively during later stages and came to ripening at about the same time as the other series. Where the silicate was added in instalments (Group V), best results were obtained as the initial harmful effects of silicate were not noticed in these cases. The yields were also definitely higher than in the corresponding Group (IV).

While the results show a similarity between silicate and phosphate treatments in that both of them increase growth and yield of grain, there is at the same time a distinct difference between the two treatments when comparing the different series. Phosphate application to dry series (manured as well as unmanured) does not increase the yield of grain so much as silicate application whereas in the " wet " series, they both raise the yield to about the same extent. Again a comparison of the relative yields in the arid and flooded series under the different groups shows that silicate application has considerably increased the yield from dry series nearer to that from flooded series than phosphate application.

The above observations are made clear from Table III, where the increase

TABLE III.

Per cent. increase over check in yield of grain and Grain/Straw ratio.

Series	Yield of Grain				Grain/Straw Ratio			
	Treatment							
	Treated with Phosphate	Treated with Silicate=S	Treated with Silicate=4S	Treated with Silicate=4S in instalments	Treated with Phosphate	Treated with Silicate=S	Treated with Silicate=4S	Treatel with Silicate=4S in instalments
	Per cent. increase over check.							
Unmanured—arid	166·7	183·2	220·9	216·8	97·5	155·9	164·3	179·4
Unmanured—flooded	194·5	147·2	139·5	181·6	130·1	123·2	93·9	144·3
Green manured—arid	130·0	201·0	224·0	232·0	16·1	32·1	36·0	46·5
Green manured—flooded	27·5	26·4	31·8	53·3	3·4	24·2	21·9	43·1

over the check (control) in the yield of grain and in the grain/straw ratio (Table II) are given for the different groups of the arid and flooded series.

Thus, the increase over check is in every case more in arid series than in flooded ones. In other words, there is greater response to growth and yield on fertilisation of arid soil with silicate. The yield of grain under arid conditions in pots treated with silicate compares very nearly with the yield of grain in flooded series.

Although the application of silicate to the unmanured soil increases the yield of grain and straw, yet it would appear from a comparison with the yields in the manured series that organic manure is the chief limiting factor governing crop growth and yield. Even in the experiments with green manure, the yield is far more in flooded series than in arid ones and as has been found earlier (Sreenivasan, *loc. cit.*), the plant absorbs larger amounts of silica in the former than in the latter cases. Since the present enquiry has shown that application of sodium silicate to manured dry series improves yield and raises it to nearly the same level as in the wet series, it would follow that the swamp soil conditions supply the plant with large amounts of silica which are otherwise unavailable. This is further supported by the fact that in the manured flooded series, silicate addition does not appreciably improve the yield. Further work is, however, needed to show whether under dry soil conditions and in the presence of a supply of soluble silica the rice plant absorbs more of silica from the soil system and whether intake of silica and yield of crop are primarily related to each other.

In the application of the principle of silicate fertilisation to the rice crop in field practice, the possible harmful effects of the silicate on the reaction and texture of the soil and on subsequent crops should not be ignored. Trials should be conducted with judicious combinations of sodium silicate with other acid fertilisers like ammonium sulphate, calcium superphosphate, etc.

Further work relating to the effect of silicate fertilisers on (*a*) the yield of dry and wet cultivated rice grown under field conditions, (*b*) the intake of silica, and (*c*) the physical properties of the soil, is under progress.

Summary.

Observations have been made on the growth of the rice plant and yield of paddy under arid and flooded conditions with and without addition of organic (green) manure, sodium silicate and potassium phosphate respectively. It is found that treatment with sodium silicate increases the yield of grain and straw and that both in unmanured and manured soils the response is greater in arid than in flooded series.

My thanks are due to Prof. V. Subrahmanyan for helpful criticism.

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FIXATION OF NITROGEN BY RICE SOILS AND RICE PLANTS.

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1. Introduction.

During the study of the nitrogen recuperation of soils, which the author is carrying on for some years past, it was observed that generally during the growing period of a crop the cropped plot showed less nitrogen than the uncropped plot but with rice lands the reverse was the case. In one instance where three crops were taken in one year it was observed that during the time the rice was growing the cropped plot had more nitrogen than the uncropped plot but as soon as other crops like Cabbage, etc., and Sweet potato came in, the cropped plot showed less nitrogen than the uncropped plot during cropping period. This naturally created a suspicion as to whether rice crop helped in some way to fix nitrogen in the soil.

It is also a well-known fact that in most of the rice lands in India sufficient nitrogen is always present to supply about 30 lbs. of nitrogen per acre annually to the crop grown on them. It seems, there is a natural fixation of nitrogen in these soils to compensate the loss due to (1) removal of nitrogen by rice crop, (2) removal of nitrogen by washing due to heavy rainfall, and (3) the loss due to decomposition of nitrates in the water-logged condition.

J. Sen (1924 and 1930) says, " An examination of the contents of roots of rice plants conducted by Dr. Harrison at Coimbatore had yielded indications of the presence of bacteria apparently capable of assimilating free nitrogen." He further says, " The development of bacterial growth in the solutions, which contained no nitrogen, clearly indicated an assimilation of nitrogen from the gaseous atmosphere.

" This demonstration of an occurrence of symbiotic nitrogen fixing organisms within the roots of the rice plant throws new light on the problem of nitrogen nutrition of rice and opens out many interesting lines of study."

B. Viswa Nath (1932) says, " An interesting observation arising from the work in Coimbatore laboratories is that there are indications that paddy plant is capable of fixing atmospheric nitrogen. This may be possible seeing

that paddy lands which have not been manured for years still maintain the yield, but the suggestion requires confirmation."

In order to ascertain the truth of the results of the previous experiments, work was done along two lines. Plot experiments were done at Karjat and Ratnagiri and laboratory experiments were carried out at Poona.

2. *Field Experiments.*

In every place a suitable area was chosen and was divided into two equal plots. The plots received the same treatment except that one was cropped and the other was not.

Samples were taken by randomisation. From each plot 12 samples of surface soil of 6" depth were taken and thoroughly mixed up and a composite sample was taken, dried at 60° C. to prevent changes and then brought to Poona. Soil samples for moisture were taken separately. The randomised spots from which the samples were taken, were distributed fairly evenly over the whole plot.

The methods of analysis were the same as those followed in the previous publications on nitrogen recuperation of soils.

Special precautions taken in analysing the samples.—

(*a*) Often investigators take 5 to 10 grammes of soil sample for nitrogen determination. 20 grammes of soil were taken to minimise errors in final figures which increase by multiplication.

(*b*) 0·05 N sulphuric acid was used for total nitrogen.

(*c*) Blank determinations were done and corrections made accordingly.

(*d*) Glass distilled water was used all through.

All these precautions were taken to minimise the working error as much as possible.

Working error.—Whatever the precautions one may take there is sure to be some working error in the case of soil nitrogen which is present in small quantities. It is essential that working error is determined to get an idea as to what extent there is real fluctuation after making necessary allowance for all sorts of errors. Soon after the work was started a soil sample was collected by randomisation of the spots, etc., on a plot. This composite sample was divided into 8 parts and soil from each of these was analysed for total nitrogen. The figures obtained were as follows :—

Milligrams of Nitrogen in 100 Grammes of Oven Dry Soil.

Serial No.	Total nitrogen
	Milligrams.
1	77·52
2	77·52
3	78·38
4	77·98
5	77·75
6	77·75
7	78·38
8	78·18

The standard deviation for total nitrogen comes to 0·3527. Any difference which is more than three times the standard deviation, *i.e.*, 0·3527 × 3= 1·0581 is significant.

1. *Experiments at Karjat.*—At Karjat on Government Experimental Farm two sets of experiments were done—one in the cold season of 1934-35 and the other in the rainy season of 1935.

(*a*) For winter season crop of rice, an area was divided into two equal plots adjacent to each other measuring 33′ × 33′ each. On one plot transplanted rice was grown and the other was left without a crop. This plot, however, received water and all other treatments just the same as the cropped plot. No plot received any manure. Ploughing and cross ploughing was given on the 10th of December. Puddling was done on the 15th and the transplanting of rice seedling was done on cropped plot on the 24th of December. The crop was harvested on the 8th of April. The plots were watered every day. The samples of soils were taken every month by randomisation and by preparing a composite sample from 12 spots from each plot. Temperatures and moistures for both the plots were nearly the same. The difference in temperatures never went beyond one degree Centigrade and the difference in moistures never beyond one per cent.

The following table gives the nitrogen month to month from the cropped and uncropped soils.

TABLE I.

Soil moistures in per cent. and total nitrogen in milligrams per 100 Grammes of oven dry fine soil.

Time	Cropped		Uncropped	
	Soil moisture	Total nitrogen	Soil moisture	Total Nitrogen
	Per cent.	Mgms.	Per cent.	Mgms.
Last week of December, 1934	32·0	78·02	30·87	78·84
January, 1935	35·71	83·80	31·02	80·53
February	28·0	76·89	27·83	73·53
March	21·78	75·19	22·5	77·83
April	4·45	75·09	4·5	74·64

(*b*) The rainy season plots were of the same size as those for the winter season and were adjacent to each other. Both of them were treated exactly alike the only difference being one carried a rice crop and the other was without one. The plots received ploughing on the 21st of June, cross ploughing on the 28th June, puddling on the 9th of July and transplanting on the 11th of July. One weeding was given on the 8th of August and another on the 19th of September. The crop was harvested on the 10th of November.

Soil samples were taken from month to month by randomisation from both the plots as in the other experiment. The variations in total nitrogen in the two plots are given in Table II below.

2. *Experiments at Ratnagiri.*—At Ratnagiri a field experiment was tried on a winter season crop of 1934-35. Two adjacent plots of one *guntha* each were taken and ploughed on the 12th of January, 1935. The plot was watered and sowing was done by broadcasting germinated seed on one plot on the 16th of January. Both the plots were watered every morning. The cropped plot was harvested on the 16th of April.

Samples of soils were taken by randomisation from both the cropped and uncropped plots. The variations in total nitrogen in the two plots are given in Table III below.

TABLE II.

Soil moistures in per cent. and total nitrogen in milligrams per 100 *grammes of oven dry fine soil.*

Time	Cropped		Uncropped	
	Soil moisture	Total nitrogen	Soil moisture	Total nitrogen
1935	Per cent.	Mgms.	Per cent.	Mgms.
Last week of July	40·22	85·53	40·22	87·86
August	40·22	85·15	40·22	82·45
September	39·85	88·49	40·07	85·30
October	33·45	85·80	32·57	83·05
November	32·64	84·49	30·79	80·71

TABLE III.

Soil moisture in per cent. and total nitrogen in milligrams per 100 *grammes of oven dry fine soil.*

Time	Cropped		Uncropped	
	Soil Moisture	Total Nitrogen	Soil Moisture	Total Nitrogen
	Per cent.	Mgms.	Per cent.	Mgms.
Second week of January, 1935	2·63	216·65	2·51	221·00
February	35·65	225·89	35·61	217·87
March	38·79	234·65	36·28	231·14
April	33·69	240·18	26·66	230·20
May	2·96	223·42	2·63	205·11

The figures for total nitrogen given in Tables I, II and III show that during the growing period of the rice crop, the cropped plot shows more

nitrogen than the uncropped plot. These results confirm those obtained in the experiments at Belgaum and Karjat given in Part IV of Nitrogen recuperation of soils mentioned in the beginning of this paper. (Sahasrabuddhe and Abhyankar, 1936.)

In all five field experiments were tried :—

(1) At Belgaum in the rainy season of 1933.
(2) At Karjat in the rainy season of 1933.
(3) At Karjat in the winter season of 1934-35.
(4) At Ratnagiri in the winter season of 1934-35.
(5) At Karjat in the rainy season of 1935.

The first two experiments are given in Part IV of nitrogen recuperation. These five experiments include three varieties of rice soils and also include rainy and winter seasons. In all the trials, total nitrogen in the cropped plots tends to be higher than that in the uncropped plots. This happens notwithstanding the fact that the rice crop, when growing, draws upon the nitrogen of the soil to build its own tissues. This is perhaps, why the rice lands have been able to maintain their fertility, although at a low level, without manuring for centuries together. Many of the rice soils have more nitrogen than other soils in the Bombay Presidency although rice soils are likely to lose more nitrogen than other soils, due to the washing action of heavy rainfall and due to the decomposition of nitrates in water-logged conditions. The maintenance of the nitrogen level of rice lands seems therefore to be due to the nitrogen fixing power of the rice soils, especially in the presence of the growing rice crop.

3. **Laboratory Experiments.*

Laboratory experiments were carried out to ascertain whether a sterilised nearly nitrogen-free solution, like that of Ashby's can fix atmospheric nitrogen (1) when rice soil is introduced in it, and (2) when rice seedlings are grown in the solution either with or without rice soil.

1. *First series.*—The first series of experiments was started on the 6th of January, 1935, and closed after ten days on the 16th of January. Nitrogens of all the contents of the flasks excepting the plants, were determined. The bacterial and other actions in the flasks were stopped on one and the same day by adding sulphuric acid to the flasks :

The results obtained are given below.

* This was submitted as a paper to the International Congress of Soil Science, held at Oxford (England) in August, 1935.

TABLE IV.

Total nitrogen in milligrams at the end of ten days in each flask.

Treatment	Nitrogen to begin with	Nitrogen after 10 days			Average of the three
		I	II	III	
1. Ashby solution	0·66	0·66	0·66	0·67	0·66
2. Ashby solution and rice root extract*		0·76	0·84	0·87	0·82
3. Ashby solution and rice root extract and rice soil (one gram)		5·24	6·29	6·94	6·16
4. Ashby solution and rice root extract and rice soil (one gram) and rice plants growing		11·46	12·86	12·98	12·43

* Roots were crushed with a small quantity of water and 5 c.c. were used.

The soil contained 0·077 per cent. of nitrogen, *i.e.*, in one gramme there was 0·77 milligram of nitrogen. The figures show that Ashby's solution by itself does not fix any nitrogen. Addition of rice root extract does not help in fixing nitrogen (the slight increase seen is perhaps due to the nitrogen in the root extract itself). Addition of rice soil decidedly adds nitrogen and the presence of the growing rice roots further increases the nitrogen contents. There is something in the rice soil which has the power of fixing nitrogen. This is further enhanced by the growing roots of the rice plants. The results of the three lots all point to the same conclusions.

2. *Second series.*—A second series was started on the 2nd of February and was closed after 10 days. To begin with all the flasks had 150 c.c. of Ashby's solution but as the flasks in which rice plants were growing showed less of liquid, 60 c.c. of Ashby's solution diluted was added to each of the flasks to have the same quantity of Ashby's solution in each. This series included the experiments done in two lots as given in the following table. The second lot had more plants than the first.

Excepting the first two experiments the duplicates do not agree with each other because the plants introduced were more in the second lot than in the first. But the trend of results is the same in both the lots. Flasks in experiment two show more nitrogen than those in experiment one because

TABLE V.

Figures for total nitrogen for the two lots are given in milligrams for each flask.

Treatment	Total nitrogen in each flask after 10 days	
	I	II
	Milligrams	Milligrams
1. Ashby solution only	1·03	1·02
2. Ashby solution and one gramme sterilised rice soil	1·82	1·82
3. Ashby solution with plants growing in solution	3·6	6·77
4. Ashby solution and one gramme air dry rice soil	9·78	10·59
5. Ashby solution and one gramme sterilised rice soil with plants growing in solution	10·22	13·26
6. Ashby solution and one gramme air dry soil with plants growing in solution ..	12·02	15·76

the soil added in the second experiment contains nitrogen which is equal to the difference in nitrogen in one and two. This shows that sterilised rice soil has no power of fixing nitrogen.

When rice plants grow in culture solution there is definite fixation of nitrogen. This is perhaps because the roots carry nitrogen fixing inoculum as the plant roots in the experiment were washed but not sterilised.

Air-dry soil introduced in the fourth experiment supplies nitrogen fixing inoculum and also a suitable medium in the form of soil. It is hence, perhaps, that good fixation of nitrogen is observed. In Experiment 5 the inoculum is introduced by the rice plant roots and a suitable medium is supplied by the sterilised soil.

In the sixth experiment there is the inoculum introduced by the plant roots as also by the air-dry soil and the medium with rice soil is quite suitable for nitrogen increase and hence the highest figures of nitrogen are given by the sixth experiment.

3. *Third series.*—In the experiments conducted so far, the rice plants were brought from rice fields at Karjat. That the Karjat soil contains the

inoculum is clearly proved by the experiments. It is however not clear whether any inoculum is carried within the seed. In order to find this out, seedlings were prepared under sterilised conditions from rice seed washed to sterilise the surface and these seedlings were then allowed to grow in sterilised Ashby solution as in the previous experiments. The Ashby solution used in this case, was reduced in strength by diluting it with three times its volume of sterilised water. This was done to give a dilute solution to very tender seedlings.

After a period of 10 days from the time the seedlings were introduced in the culture flasks, the nitrogen fixed as determined in each case is given below.

TABLE VI.

Milligrams of total nitrogen in each flask after ten days.

Treatment	Total nitrogen in each flask	
	I	II
	Milligrams	Milligrams
1. Ashby solution only	0·81	0·81
2. Ashby solution with plants from sterilised seed	1·05	0·96
3. Ashby solution and one gramme sterilised rice soil with plants from sterilised seed ..	1·88	1·70
4. Ashby solution and one gramme air-dry rice soil with plants from sterilised seed ..	3·59	3·41

The results of the duplicates agree with each other. The slight excess of nitrogen of the second experiment over that of the first must be due to small quantity of root material dropped in the culture during washing, etc. The excess of nitrogen in three over that in two is due to the nitrogen in the sterilised soil. It therefore clearly shows that no nitrogen fixing inoculum is carried within the rice seed. The fourth experiment shows definite fixation of nitrogen and this must be put down as due to the inoculum in the air-dry rice soil.

It can, therefore, be safely stated that nitrogen fixing inoculum is not carried within the rice seed although it may be carried on the surface of the seed. On the other hand it is sure that the inoculum is in the rice soil and

may be carried along with the soil. The presence of growing rice roots helps this inoculum in fixing larger quantities of nitrogen than the soil would otherwise fix because the rice roots form a good host.

4. Conclusion.

It was observed in the previous experiments done in connection with the nitrogen recuperation of soils that the cropped rice plot shows more nitrogen than the uncropped rice plot during the growing period of the rice crop. These results have been confirmed by the field experiments, in the winter season at Karjat and Ratnagiri and in the rainy season at Karjat.

These results received further confirmation by the experiments done in the laboratory which show that the rice soils have the power of fixing nitrogen and this fixation is helped by the presence of the growing roots of the rice plant. It has been shown that the rice seed does not carry within it any nitrogen fixing organisms.

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INVESTIGATIONS ON THE RÔLE OF ORGANIC MATTER IN PLANT NUTRITION.

Part XII. Production of Organic Acids during Decomposition of Cane Molasses in the Swamp Soil.

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(Communicated by Prof. V. Subrahmanyan, D.Sc., F.I.C.)

It was shown in a previous communication (Bhaskaran, Narasimhamurthy, Subrahmanyan and Sundara Iyengar, 1934) that the more important products of decomposition of cane molasses in the swamp soil are (*a*) lactic acid, (*b*) volatile fatty acids, chiefly acetic, propionic and butyric acids, (*c*) traces of ethyl alcohol and acetaldehyde, and (*d*) gaseous products comprising chiefly of carbon dioxide, methane and hydrogen. The later work of Narasimhamurthy (under publication) showed that the gaseous products account for only a small part of the added organic matter. Narasimhamurthy and Subrahmanyan (1935) observed that the carbonaceous products are mostly water soluble and are largely present in the supernatant. Sundara Iyengar and Subrahmanyan (1935) found that the minerals—especially iron and aluminium—which are brought into solution during the fermentation of carbohydrates are mostly associated with organic acids and not with carbon dioxide as suggested by some previous workers. These and other observations would point to the conclusion that organic acids are the chief products of decomposition and are responsible—directly or otherwise—for the more important changes associated with the application of cane molasses as a fertiliser.

The study of the production of organic acids is of considerable scientific as well as practical interest. It has already been shown by Subrahmanyan (1929) that organic acids are produced by a variety of carbohydrate materials, so that and since most organic manures are derived from carbohydrate materials, it may be assumed that they will accompany the application of almost every type of organic manure to the swamp soil. There is evidence to show that in addition to facilitating increased dissolution of calcium, potassium and other ingredients, they also help in important biochemical processes such as nitrogen fixation. The recent observations of

Bhaskaran and Subrahmanyan (1935) would indeed show that even when a carbohydrate is applied to the soil, the non-symbiotic nitrogen fixers utilise, chiefly, the organic acids which are the immediate products of decomposition.

In view of the above, the present enquiry on the production and distribution of organic acids was undertaken.

Experimental.

Materials.—The soil used in the present study was a red, sandy loam of the type commonly met with in many parts of the Deccan plateau. Its chemical composition has been discussed in a previous communication (Bhaskaran *et al., loc. cit.*). The molasses used was a solidified product, the composition of which has also been given in the publication under reference.

Samples of air-dry soil were weighed out into suitable glass containers and molasses (in concentrated solution) added in quantities corresponding to 1 per cent. on the weight of the soil (corresponding to 10 tons per acre). The soil was treated with 2½ times its weight of water, the containers plugged with cotton wool and the suspensions incubated at 37°.

At stated intervals, representative specimens were removed and analysed for the various acids. The determinations were carried out on separate lots, each of which contained 300 g. of soil and the corsesponding amounts of molasses and water. The acids present in water soluble form (free as well as combined) were first removed by filtering. The residue was treated with phosphoric acid (20 c.c., 10 per cent.) and water and then refiltered, followed by liberal washing. The two extracts thus obtained were mixed together and then made up to volume. Aliquots were then taken for subsequent analysis.

Methods : *Estimation of lactic acid.*—The procedure described by Subrahmanyan (1929) was followed. A number of preliminary trials were carried out, adding known quantities of lactic acid to soil suspensions containing glucose and it was found that, in all the cases, accurate estimates of the added acid could be obtained. Volatile fatty acids did not interfere with the estimation.

Total volatile fatty acids.—Even prolonged steam distillation yielded low and discordant values. Vacuum distillation, as recommended by Birkinshaw and Raistrick (1931), was therefore followed with satisfactory results (Table I).

Individual volatile fatty acids.—A number of samples of soil suspensions, representing different stages of decomposition, were filtered and the filtrates steam-distilled. The individual acids present in the distillates were detected

TABLE I.

Volatile fatty acids (in terms of c.c. N/10 alkali).

Taken	As estimated by	
	Steam distillation	Vacuum distillation
15·75	12·15, 11·20, 11·80	15·60, 15·50, 15·60
31·50	29·70, 30·05, 28·95	31·45, 31·40, 31·50

by the orientation tests described by Dyer (1916). The same acids—acetic, propionic and butyric—were found to be present at all stages. The quantitative estimations were carried out according to the Duclaux method as modified by Gillespie (1917).

The procedure though rather tedious yielded more accurate results than the steam distillation method. The accuracy of the results was not affected

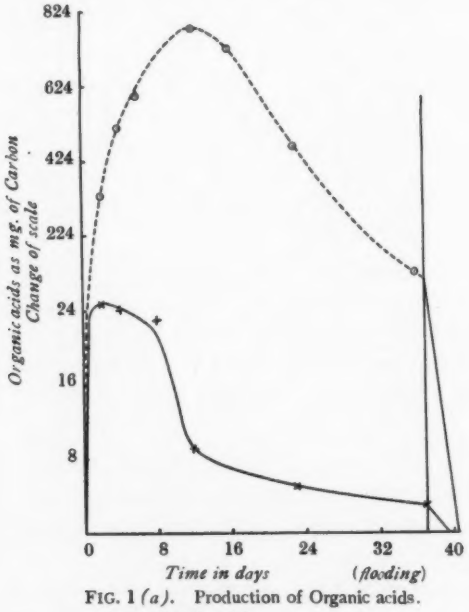

FIG. 1 *(a)*. Production of Organic acids.
Lactic acid
Total volatile fatty acids

by the presence of sugars and other non-volatile substances present in the medium.

The samples were first taken out once in two days, and then at longer intervals. After 37 days the soils were flooded and the water solubles decanted out from time to time. Two sets of samples were then examined for their acid contents. As the quantities thus found were almost negligible further analyses of the flooded samples were not carried out. The results have been presented in Figs. 1 (*a*) and 1 (*b*).

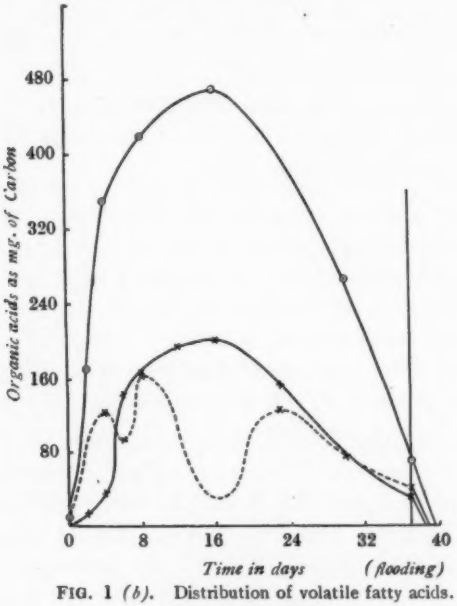

FIG. 1 (*b*). Distribution of volatile fatty acids.
Acetic acid
Propionic acid
Butyric acid

It may be observed that there was maximum production of lactic acid in the course of the first two days. After that period, the quantity tended steadily to diminish. The most rapid fall was noticed between the 6th and the 12th days when over 17·8 mg. (as equivalent of carbon) were lost. Only a very small amount was present on the 37th day, but even that was completely removed by flooding.

The volatile fatty acids were always present in very much larger quantities than lactic acid and accounted for the bulk of organic acids present in the medium. The quantities were comparatively small in the early stages, but they increased steadily, until on the 12th day they were at a maximum.

They then decreased rapidly, until on the 37th day they were present only in small quantities. Even that was largely washed out on flooding. The volatile and the non-volatile acids, taken together, would account for the major part of the carbon originally added as molasses.

Time in days	2	4	6	8	12	16	23	30	37	44
Total acids (volatile and non-volatile) as percentage of added carbon ..	42·3	61·1	70·0	84·9	81·6	76·8	50·5	51·6	15·7	6·7

It would indeed be found that after deducting the carbon lost as gas (chiefly carbon dioxide and methane), the acids would represent about the entire bulk of the residual organic matter.

Attention has already been drawn (Bhaskaran, Narasimhamurthy, Subrahmanyan and Sundara Iyengar, *loc. cit.*) to the fact that the sugar of the molasses disappears completely in the course of the first three days. As the maximum production of volatile fatty acids was not reached until the 12th day, it has to be inferred that, at any rate, a large part of the fatty acids was not derived from the sugar itself, but from some other substance which was an immediate product of the decomposition.

The quantities of the volatile fatty acids present at different stages were very much in excess of the corresponding amounts of lactic acid, so that it does not appear probable that the former were derived from the latter. It is not unlikely, however, that the production of lactic acid at each stage was followed immediately by its decomposition, so that the quantity of that acid actually present at any time was comparatively small.

It may be observed that the quantities of acetic and butyric acids increased steadily upto the 16th day after which they rapidly declined. Propionic acid followed a different course. There was a fairly useful quantity in the beginning, but subsequent changes are comparatively small. These observations would suggest that the agency (or agencies) responsible for the production of acetic and butyric acids were more or less similar, while that yielding propionic acid functioned in a different manner. The mechanism of the decomposition of organic acids after the first three weeks is still obscure. A small part would, no doubt, have been oxidised to carbon dioxide. In the light of the previous observations of Narasimhamurthy and Subrahmanyan (*loc. cit.*), it has to be inferred, however, that the major part remained in the medium in some other water soluble form.

Transformations of lactic acid and alkali lactate into volatile fatty acids.— With a view to determining whether under swamp soil conditions, lactic acid can be converted into volatile fatty acids, and, if so, whether the distribution of the acids is the same as that accompanying the decomposition of molasses, some experiments were carried out adding (*a*) glucose (1·5 g.); (*b*) lactic acid (syrupy acid, 1·5 g.); (*c*) sodium lactate (equivalent to 1·5 g. of lactic acid); (*d*) lactic acid (same as in *b*, 1·5 g.) *plus* glucose (0·03 g.); and (*e*) sodium lactate (same as in *c*, 1·5 g.) *plus* glucose (0·03 g.) respectively to suspensions containing 300 g. of soil in 750 c.c. of water. One set (*f*) of soil suspension was also left untreated as control. The samples were incubated at 37° for 8 days after which they were analysed for volatile fatty acids. The results have been given in Table II.

TABLE II.

Distribution of volatile fatty acids in mg. of carbon.

	Acetic	Propionic	Butyric	Molecular Proportion (Ac : Pc.)
(*a*)	50·4	61·2	139·2	1 : 1·2
(*c*)	60·0	205·2	96·0	1 : 2·3
(*e*)	74·4	288·8	105·6	1 : 2·0

The distribution of acids in *b*, *d* and *f* have not been recorded as the quantities were very small.

It may be seen that in only three of the cases was there fairly large production of the fatty acids. These contained, originally, either glucose or sodium lactate alone or a mixture of the two. In two of the others, the quantities of acid produced were comparatively small, while, in the third one, it was almost negligible : the former contained lactic acid with or without minute quantities of glucose, while the latter was the untreated control. Although the three fatty acids were present in all the cases, the total quantities, as well as the proportions, differed with the nature of the treatment. With glucose alone the total quantity of acids was only about two-thirds of that formed from sodium lactate. In the former case, acetic and butyric acids formed the chief products while propionic acid accounted for only about a fifth of the total : in the latter, propionic acid formed the major product while acetic and butyric acids were present in smaller proportions. The proportion of acetic to propionic acid as derived from sodium lactate was approximately as 1 : 2. This is in accordance with the Fitz

equation

$$3\ CH_3\ CHOH\ COOH \rightarrow 2CH_3 \cdot CH_2 \cdot COOH + CH_3COOH + CO_2 + H_2O$$

and in agreement with Virtanen's theory (*loc. cit.*) of fermentation. It may be concluded from the above that the production of acids from sugar did not follow the same course as that from sodium lactate. These observations, combined with the previous findings, would show that (*a*) the volatile fatty acids were mostly derived from some intermediate (non-reducing) substance which was the immediate product of decomposition of sugar, and (*b*) lactic acid was either not used at all or, at any rate, only to a small extent in the production of the fatty acids.

It is probable that the comparatively poor production of fatty acids from suspensions containing lactic acid was due to the increased H-ion concentration of the medium. On neutralising the acid, the rate of fermentation and the attendant production of fatty acids were greatly accelerated.

Further work relating to the influence of various factors such as the nature of the soil, concentration of molasses, temperature and degree of submergence on the production and distribution of organic acids is in progress and will be the subject of a later communication.

Summary.

1. Lactic, acetic, propionic and butyric acids are the chief products of fermentation of cane molasses in the swamp soil. Of these, acetic and butyric acids form the major part, while propionic and lactic acids are present in smaller quantities. There is no evidence to show that the volatile fatty acids are derived from lactic acid.

2. Fermentation of sodium lactate by the mixed flora of the soil results in the production of all the three volatile fatty acids. Propionic acid is the chief product and its molecular proportion is about twice that of acetic acid. Butyric acid is present in still smaller quantities.

3. Fermentation of lactate in the soil proceeds in accordance with the Fitz equation and the Virtanen theory of fermentation; that of molasses follows a different course.

The author's thanks are due to Prof. V. Subrahmanyan for his kind interest in the progress of the work and helpful criticism.

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INDIAN WATER-MOULDS.—II*

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1. *Achlya prolifera* (Nees). de Bary, *Bot. Zeit.*, 1852, **10**, 473, Pl. 7, Figs. 1–28. (Plate I.)

Growth dense and stout, reaching a length of 1. 45 cm. on egg-yolk in distilled water. Primary zoosporangia cylindrical, terminating the stout hyphæ. Secondary zoosporangia rare; when present, arising in a cymose manner from below the primary ones. Oogonia in racemes on the ends of lateral branches of the main hyphæ, spherical. Oogonial wall pitted. Eggs variable in number, upto 8 in an oogonium, eccentric. Antheridial branches diclinous, much twisted and branched, winding like a parasite about the oogonia. Gemmæ formed by the segmentation of the hyphæ.

Growth in culture.—On egg-yolk in distilled water—growth dense, upto 1·45 cm. in length, oogonia plentiful. On egg-white in distilled water—growth strong, zoosporangia plenty, oogonia few. On boiled corn grain in distilled water—growth luxuriant, abundant gemmæ, zoosporangia in profusion, but no oogonia.

Collected from Lahore and Sheikhupura.

It agrees with de Bary's description of the fungus.

2. *A. klebsiana* var. *indica*, nov. var. (Plate II.)

Growth moderately stout, reaching about 1·6 cm. on egg-yolk in distilled water. Hyphæ branched, tapering gradually towards the apex. Zoosporangia 25·2–60 μ × 180-300 μ. Secondary ones not very common, when present arising by cymose proliferation. Oogonia plentiful, borne on lateral stalks, which are from equal to, to twice the diameter of the oogonium; spherical, 36-70.8 μ in diameter; wall of the oogonium unpitted, oogonial neck very small. Eggs variable in number, 3-14, commonly 2-4; 12-30 μ in diameter, mostly 19.6-25·2 μ; spherical, eccentric. Antheridial branches slender, always diclinous, rarely branched. Gemmæ formed by the segmentation of the hyphæ; dense and irregularly rod-like, attenuating at the tips or ending in a knob.

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* In continuation of Indian Water-Moulds.—I. By H. Chaudhuri and P. L. Kocchar. Published in *Proc. Ind. Acad. Sci.*, 1935, **2**, No. 2, Section B.

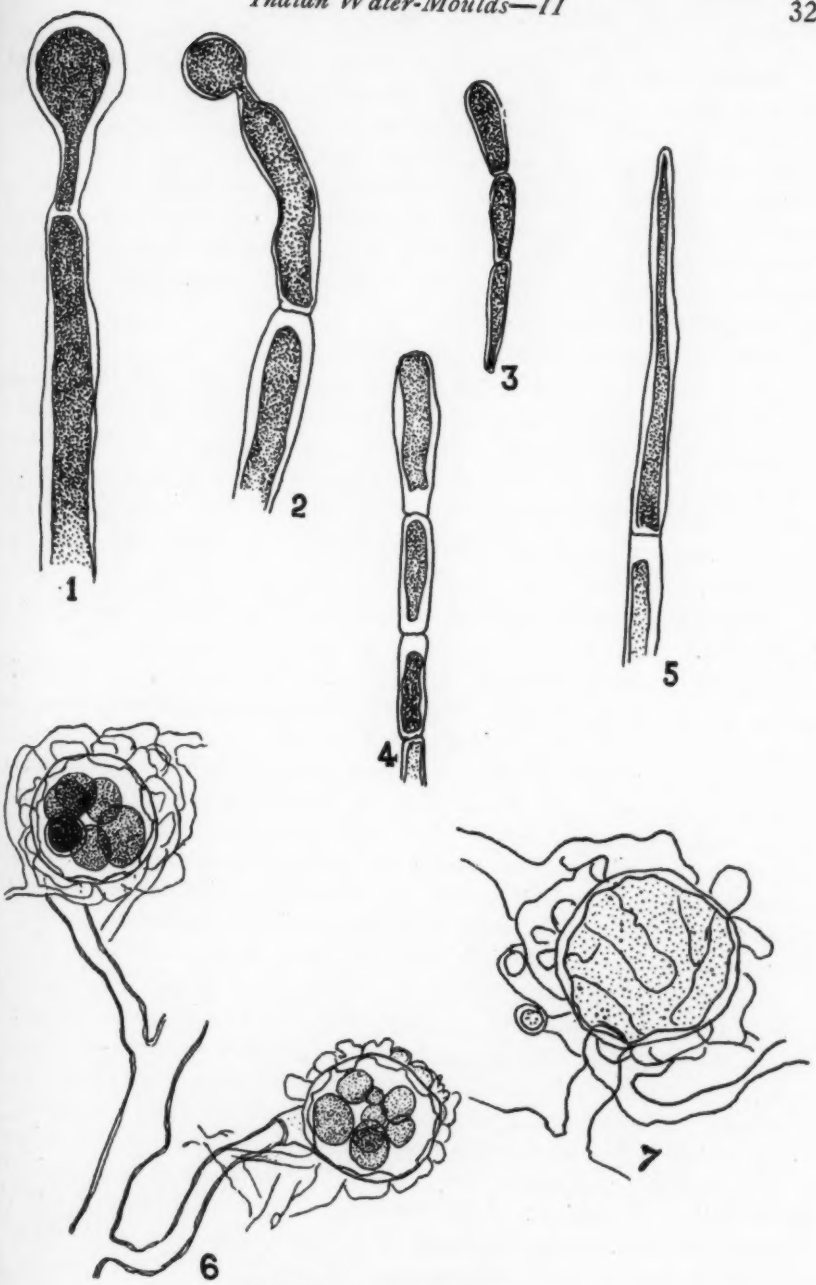

PLATE I.—*Achlya prolifera.*

Growth in culture.—On egg-yolk in distilled water—growth fairly stout, reaching about 1·6 cm.; oogonia plentiful, but not so antheridia. On egg-white in distilled water—vegetative growth delicate, hyphæ slender, oogonia present, with antheridia on less than 40% of them. Zoosporangia formed. A culture on house-fly in distilled water, which developed normal oogonia, produced oogonia more plentifully but very few gemmæ when transferred to ·1% solution of leusin. A culture on an insect formed zoosporangia which developed zoospores, some of the zoosporangia liberating the zoospores. No oogonia.

Collected from Mughalpura and Calcutta.

A. klebsiana var. *indica* differs from the main species in the size and form of the zoosporangia which in this case are smaller and thicker and in developing comparatively few secondary zoosporangia; in the shorter neck of the oogonia; in the larger number of the eggs in an oogonium and in the form of the gemmæ which may attenuate at the tips.

3. *Thraustotheca clavata* (de Bary) Humphrey, *Trans. Am. Phil. Soc.*, 1892 (1893), **17**, 131.

Dictyuchus clavatus de Bary, *Bot. Zeit.* 1888, **46**, 649, Pl. 9, Fig. 3. (Plate III.)

Growth luxuriant, reaching a length of 1.89 cm. on corn grain in distilled water. Hyphæ stout, straight, branched, 20·4-78 μ in thickness, averaging 36 μ; branches of the hyphæ much curved and twisted. Sporangia terminal, usually short, broad and clavate, sometimes bearing resemblance to those of *Pythiopsis*, 36-78 μ × 66-240 μ. Secondary sporangia not formed. Spores variously shaped, while inside the sporangia. Spores liberated by the fragile sporangial wall breaking down. Oogonia borne on short, straight lateral stalks; spherical, 31·6-69·6 μ in diameter. Wall smooth or slightly pitted; oogonial neck prominent. Eggs 1-6 in an oogonium, 18-24 μ in diameter, eccentric. Antheridia diclinous, many to an oogonium.

Growth in culture.—On boiled corn grain in distilled water—growth luxuriant, sporangia plentiful, but no oogonia. On egg-yellow in distilled water—growth strong with sporangia and oogonia. On egg-white—as on corn grain. On an insect in distilled water—growth delicate with only a few sporangia. A culture on house-fly in distilled water, when transferred to 0·1% leusin solution developed oogonia in two weeks. A culture on an insect in distilled water, when transferred to 0·1% solution of potassium nitrate, was retarded in its growth. A culture on egg-yolk, when transferred to 0·1% solution of leusin, produced normal sporangia and a good many oogonia.

Collected from Dakha, Ludhiana.

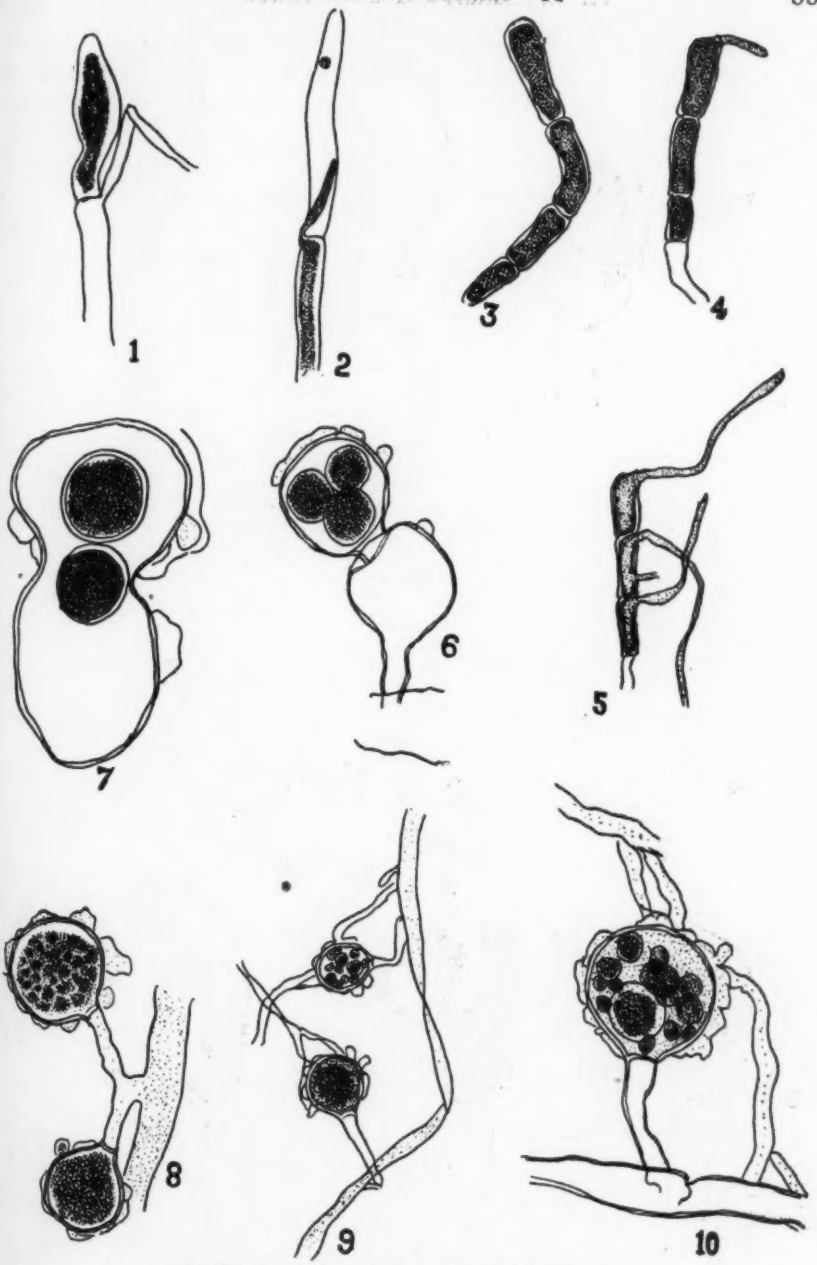

PLATE II.—*Achlya klebsiana* var. *indica*, n. var.

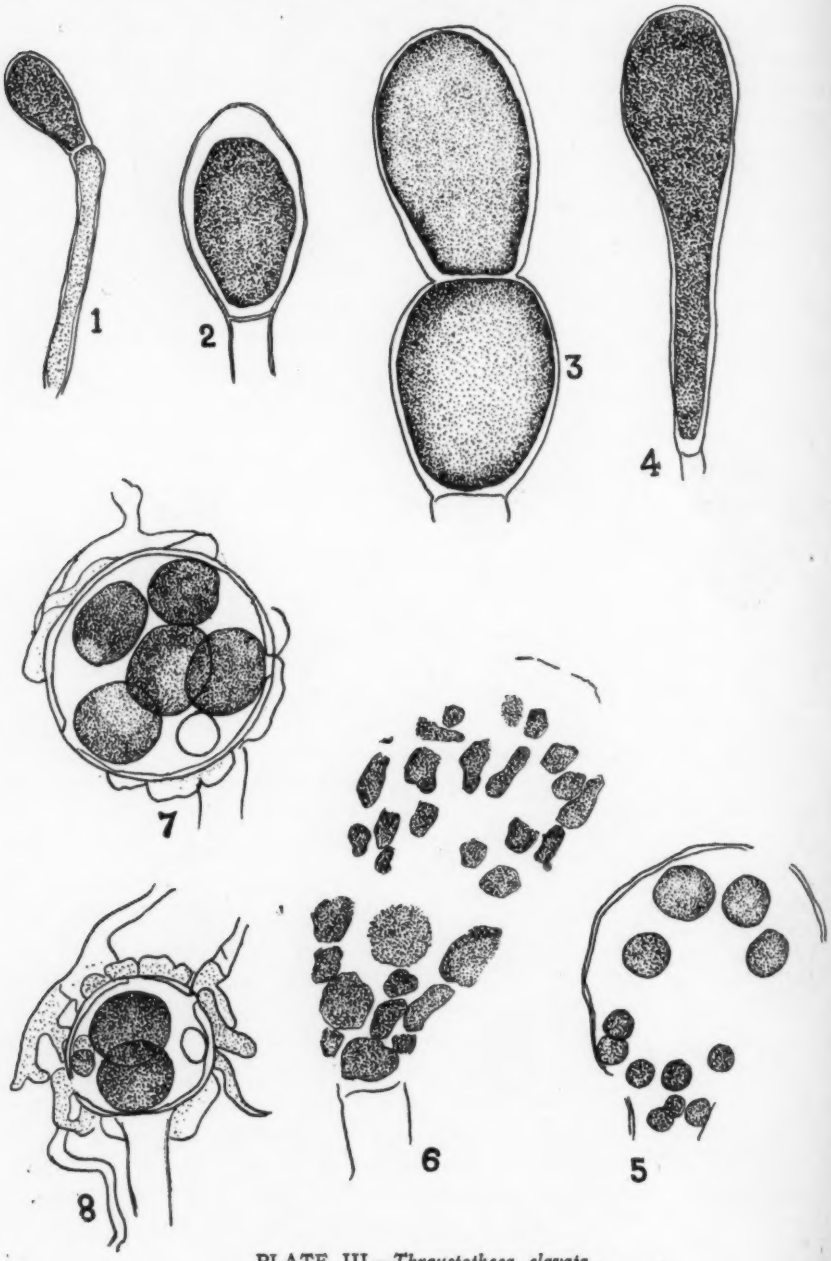

PLATE III.—*Thraustotheca clavata.*

The species is characterised by the form and size of its sporangia and differs from the rest of the water-moulds in the method of spore-liberation, which takes place by the breaking down of the sporangial wall. The absence of the secondary sporangia is also characteristic.

EXPLANATION OF PLATES.

PLATE I.

FIGS. 1, 2—Zoosporangia changing at the tips into oogonia. (1) × 400, (2) ×140.
FIGS. 3, 4—Gemmæ formed by segmentation of the hyphæ. (3) × 100, (4) ×150.
FIG. 5—Zoosporangium. × 200.
FIGS. 6, 7—Oogonia surrounded profusely by antheridia. (6) × 400, (7) ×640.

PLATE II.

FIGS. 1, 2—Zoosporangia showing proliferation. × 160.
FIGS. 3, 4—Gemmæ. × 160.
FIG. 5—Germinating gemmæ. × 160.
FIG. 6—Double oogonium. × 430.
FIG. 7—A notched oogonium with two eggs and diclinous antheridia. × 640.
FIG. 8—Two oogonia with a common stalk and diclinous antheridia. × 400.
FIG. 9—Oogonia showing androgynous and diclinous condition of antheridia. × 160.
FIG. 10—Androgynous and diclinous condition of an oogonium shown in detail. × 400.

PLATE III.

FIGS. 1, 2—Young sporangia. (1) × 100, (2) × 420.
FIG. 3—A double sporangium. × 420.
FIG. 4—An elongated sporangium. × 420.
FIGS. 5, 6—Two ruptured sporangia with scattered mass of spores. (5) × 660, (6) × 1000.
FIG. 7—An oogonium with five mature eggs. × 660.
FIG. 8—An oogonium with diclinous branched, stout and crooked antheridia. × 420.

THE PHYSIOLOGY OF INDIAN NODULE BACTERIA.

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Chapter I.

Introduction.

It was theoretically a scientific assumption that, under the special conditions of Indian soils and host plants, such adaptable organisms as bacteria might exhibit certain variable peculiarities which could give us a better insight into their nature; perhaps also certain phenomena could be better observed under the laboratory conditions naturally prevailing in our country and acquire the full significance; perhaps even certain features were obscured and certain potentialities completely hidden by more conspicuous phenomena observed in foreign laboratories.

This surmise, which was merely reasonable, proved in fact also correct, and the result of our work is that we are now able to assert certain characteristics about our Indian strains without having merely to rely on analogies; and on the other hand we have added in some places new information about the organisms studied.

In addition, we provide for future workers a reliable techniqu ecovering all the ground of the physiological study of nodule bacteria which was developed after long and careful study of the methods available and which in our country was found to succeed where other methods, apparently successful abroad, had failed.

Summary of the Method.

I. The first step was the isolation of the micro-organism in pure culture. For that, three typical host plants were chosen. It was thought advisable to select three leguminous plants of economic importance in India and indigenous to the Old World. For purposes of more accurate comparison the experiments on the three isolated organisms should be carried on at the same time.

The groups according to the latest work done (Baldwin and Fred, 1929) in this matter are:—

(*a*) *Rhizobium leguminosarum* Frank. The organism causing the formation of nodules upon the roots of *Lathyrus, Pisum, Vicia* and *Lens.*

(*b*) *Rhizobium trifolii* Dangeard. The organism causing the formation of nodules upon the roots of *Trifolium* sp.

(*c*) *Rhizobium phaseoli* Dangeard. Causes the formation of nodules on *Phaseolus vulgaris, Ph. angustifolius* and *Ph. multiflorus.*

(*d*) *Rhizobium meliloti* Dangeard. The organism causing the formation of root nodules upon *Melilotus, Medicago* and *Trigonella.*

(*e*) *Rhizobium japonicum* (Kirchner) Comb. Nov. The organism causing the formation of root nodules on *Soja max.*

The plants chosen were *Cajanus indicus, Dolichos biflorus* and *Psophocarpus tetragonolobus* (Hooker, 1874). The isolation and identification of the micro-organism was made to satisfy all the microscopical and morphological data as well as the typical reactions ; and was always counterproved by the biological positive and negative test, as will be seen later.

Once obtained in pure culture, we proceeded to study the organism in question using all pertinent culture media suggested by the experience obtained during the last twenty years of microbial research.

The physiology of a micro-organism, the growth, the colonial aggrupation, the rate of reproduction,are all dependent on the nature and the variety of the enzymes, and these are tested on carefully selected media of varying composition.

The micro-reactions are sometimes made more evident by the use of indicators. This study occupied the body of our work and the results are given below.

II. A remarkable fact which we encountered in the progress of our work is that in the case of *C. indicus* we isolated, quite consistently, from the root nodules two organisms. The typical symbiont and another one, referred to below, which was not able to produce nodules, when inoculated into a pot in which the plant from which it was isolated was grown under sterile conditions.

The typical symbiont was able to produce nodules not only on the same plant from which it was isolated, *i.e., C. indicus* but also on others on which we tried it, *viz., D. biflorus, Ps. tetragonolobus* and even cowpea (*Vigna catiang*).

III. The organism of *Cajanus indicus* has been placed by Löhnis and Leonard, 1926, and others in the cowpea group, a group which is considered to have an alkaline reaction on litmus milk with the formation

or not of serum zone. But in our experiments we found that the organism of *Cajanus indicus* does not behave as those of the cowpea group. In all the experiments we found that the organism in question does not give an alkaline reaction. On the contrary it is distinctly and consistently acid.

Also in its fermentation reactions we found that it deviates from the reactions of the organisms of the cowpea group as will be mentioned later.

Various attempts were made to grow plants in flasks as recommended by Garman and Didlake ; but, although the plants did grow and develop to a stage in which nodules could be expected, we never observed actual nodule formation, even when modifying in different ways the conditions of the experiment.

IV. As regards pot experiments it was found that the usual method of growing the plants in open pots did not give consistent results.

As contamination cannot always be avoided with this method, it is always to be feared. The technique of protecting the pots with gauze, providing at the same time glass tubes as outlets for the plants and a test tube opened at both ends as an inlet for watering, proved very successful.

Isolation.

In order to isolate the organism from the nodules in the beginning of our work the method generally followed by the other workers was adopted.

A few good-sized and apparently-sound nodules were selected and cut off the roots leaving a few millimetres of the root on both sides in order to make the handling with forceps easier and also to keep the nodules intact, thus preventing the disinfecting fluid from penetrating them. The nodules were then washed several times in distilled water to remove the soil particles adhering to them. They were then sterilised by putting them into the following sterilising liquid :—

Mercury Bichloride	.. ..	1 gm.
Hydrochloric Acid	.. ..	2·5 c.c.
Water	.. ..	500 c.c.

The nodules were well shaken in the above solution for 3–4 minutes. Then they were transferred aseptically to a sterile dish containing sterile distilled water. They were washed 3–4 times, dried on a sterile blotting paper, dipped into 95% alcohol, and then passed quickly through the flame to remove the last traces of alcohol. A fair-sized nodule was transferred to a flamed glass slide and cut open aseptically. The central soft portion of the exposed surface was removed on to a few drops of sterile water with an arrow-headed needle. This was well shaken to make an emulsion.

A little of this emulsion was transferred to a medium tube and plated (Giltner and others).

This in the first place is a long and tedious process; that, however, would not have been considered as an obstacle if good results were obtained to compensate for the efforts, but as a matter of fact time and again it produced negative results.

This difficulty has been experienced by all the workers (Joshi in our country amongst others). Therefore an attempt was made to derive more light from their experience and develop a suitable standard method, at least a standard method which could be considered as satisfactory for our study.

The nodules, after being washed in ordinary tap water, were put into a sterile test tube with a cotton plug. A few c.c. of the above disinfecting fluid were added to the tube containing the nodules. The plug was replaced and the tube was put in an Erlenmeyer vacuum flask and connected to the exhaust pump. The pump was worked to exhaust the air. This step is taken in order that the disinfecting fluid may reach the entire surface of the nodule. The whole procedure does not take more than 2–3 minutes with a good "Genco" pump. The nodules that were at the bottom of the solution would float when the air was exhausted. The flask was disconnected and the tube was taken out of it. The plug and the mouth were flamed, and the mercuric chloride thrown away, care being taken that the nodules did not pass out. Sterile water was added to the tube, it was plugged and the same process of exhausting the air repeated, so that the last traces of mercury bichloride were removed as recommended by Hass and Fred (1919).

The nodules were washed with sterile water 4–5 times. As a measure of control of future operations, before an emulsion was made, we always plated out the last wash; by which we had perfect assurance that no other soil organisms were present. The presence of an occasional air or water organism is of course immaterial. It was found generally that this fourth or fifth wash was sufficient, as the rinsing liquid did not show any growth. The nodules were then broken inside the tube and an emulsion made. A drop of the emulsion was further diluted and plated out. By following the outlined procedure we always obtained good growth of colonies and free from undesirable contamination.

For the actual isolation and culture of the specific organism various media were tried. From that we gained a fairly good, and certainly interesting knowledge of conditions of growth, at least in this country.

As many times the success of laboratory procedure depends on small points which are likely to be taken for granted in routine work, we give in some detail below, not only the formula but also, where necessary, the way in which we prepared the different culture media.

Instead of using 1·5% agar so commonly recommended by the various authors, we generally use 2% agar as 1·5% agar does not give a good solid medium under our conditions of temperature and in our case also of pH. (This is otherwise the general practice in our Laboratory.) However, as this organism often produces gum it is advisable to work with a somewhat harder medium and therefore the percentage of 2·5 was found to be the best and was generally adopted for critical work. Besides the condition of the medium may also create difficulty in the passage of slant sub-cultures, when it is impossible to do any approximate comparative work by means of the loopfuls unless the slants offer a smooth and firm surface.

Whenever the agar is available in powder form we recommend soaking it in water for half to one hour before heating it. If the agar is heated in water at once, without previously soaking it in water, as mentioned before, clots will invariably be formed and sometimes even charring may take place. After soaking the agar in water it should be heated on an asbestos gauze and boiled for at least half an hour and after that it is made to the original volume by the addition of distilled water. Then the ingredients are added and the resulting preparation is heated on the water-bath to obtain perfect solution. The pH was adjusted with normal sodium hydroxide and the media tubed in 5 c.c. amounts for the slants and 12 c.c. for the plates and autoclaving was carried out at one atmosphere pressure for half an hour. The glass material used was always Pyrex, Jena or another kind of neutral glass. We cannot too strongly recommend the use of special glass when critical results are aimed at in microbial research, specially with nodule bacteria. As stated above the ingredients, specially carbohydrates, were added last, in order to avoid decomposition by prolonged heating. Moreover, the tubes were always placed in the autoclave when the steam was beginning to pass out freely through the tap. Another precaution taken to avoid decomposition of sugars was to remove the tubes from the autoclave as soon as the pressure went down and to transfer them to the refrigerator. By this method we found that the sugars did not dissociate and no turbidity occurred, the media thus remaining quite clear. If the above precaution was not taken it was very difficult to avoid turbidity. The slants were never used immediately. They were at least kept for 2–3 days in the incubator before being used, so as to be sure of their being sterile.

At no time in our procedure were media allowed to remain for more than 20 days. We found that on standing for a long time, even in the refrigerator, the pH of the media used to vary considerably. Moreover, the media lost much of their water and became so dry that no growth could take place when inoculated.

Coming to the particular media the following data will give enough information. A comparative comment on the results will be given when the description of the methods have been dealt with.

Lipman and Fowler's Synthetic Agar.

Dextrose	10·0 gm.
Potassium hydrogen phosphate	0·5 ,,
Magnesium sulphate	0·2 ,,
Potassium nitrate	0·05 ,,
Agar	20 to 25 ,,
Distilled water	1000 c.c.

Winogradski's Medium.

Distilled water	1000 c.c.
Agar	20 to 25 gm.
Sodium asparaginate	1·0 ,,
Magnesium sulphate	0·2 ,,
Glucose	1·0 ,,
Ammonium dihydrogen phosphate	1·5 ,,
Calcium chloride	0·1 ,,
Potassium chloride	0·1 ,,
Ferric chloride	Trace

Barlow's Ash Maltose Medium.

Wood ashes	16 gm.
Maltose	10 gm.
Distilled water	1000 c.c.

his medium was prepared as follows:—

Wood ashes were mixed with water and boiled for one minute. Filtered through two sheets of filter paper. The filtrate was mixed with agar and sugar and heated till dissolved. It was filtered through cotton wool, tubed and sterilised.

Wood Ash Extract Sucrose Medium.

Wood ashes	15·0 gm.
Mono-potassium dihydrogen phosphate	3·0 ,,
Sucrose	10·0 ,,
Water	1000 c.c.

Greig Smith's Levulose Agar, 1912.

Levulose	20·0	gm.
Asparagin	0·6	,,
Sodium citrate	1·0	,,
Potassium citrate	1·0	,,
Agar	25·0	,,
Distilled water	1000	c.c.

Bean Extract Medium.

In the preparation of bean extract medium, after trying the modifications of Löhnis (1930), we did not follow them as the procedure is a long and tedious one and is not justified by striking results. It was found that the original method of Maze (1897) gave as good results as the modification by Löhnis. Nevertheless, we have to note that we made a change in Maze's method, *viz.*, instead of heating the haricot beans for half an hour at 100° C. we heated them with a large quantity of water on direct flame for 2 to 3 hours by which the maceration is affected and the availability of the starch material considerably influenced. As the volume of water and the weight of beans was not specified, we took 200 gm. of seed for one litre of extract. Generally three litres of water were added and the infusion heated as mentioned before for 2 to 3 hours. The extract was passed through a strainer and made up to a litre. To the extract 2·5% agar was added and the medium prepared in the way described in the beginning. 2% of sucrose was added to this medium. Occasionally calcium carbonate was also added, but very often we prepared this medium without calcium carbonate as it served quite well.

Laurent's Sucrose Medium.

Tap water	1000·0	c.c.
Sucrose	10·0	gm.
Mono-potassium dihydrogen phosphate	1·0	,,
Magnesium sulphate	0·5	,,
Agar	20–25	,,

Soil Extract Mannite Agar.

Soil Extract	1000·0	c.c.
Mannite	20·0	gm.
Di-potassium monohydrogen phosphate	0·5	,,
Agar	20–25·0	,,

Soil extract was prepared by heating one kilogramme of rich garden soil in 1500 c.c. of water for half an hour in the autoclave.

Marmite Mannite Medium (*Desai*).

Marmite	2·0	gm.
Mannite	20·0	,,
Di-potassium monohydrogen phosphate	0·5	,,
Magnesium sulphate	0·2	,,
Agar	20 to 25·0	,,
Distilled water	1000·0	c.c.

Calcium carbonate added if desired.

Ashby's Mannitol Agar (*modified*).

Distilled water	1000·0	c.c.
Mannitol	12·0	gm.
Mono-potassium dihydrogen phosphate	0·2	,,
Magnesium sulphate	0·2	,,
Sodium chloride	0·2	,,
Calcium sulphate	0·1	,,
Calcium carbonate	5·0	,,

Yeast Water Mannitol Agar.

Agar	20–25·0	gm.
Mannitol	10·0	,,
Di-potassium monohydrogen phosphate	0·5	,,
Magnesium sulphate	0·2	,,
Sodium chloride	0·1	,,
Calcium carbonate	3·0	,,
Distilled water	900·0	c.c.
Yeast water	100·0	,,

Yeast water was obtained by soaking 10 gms. of yeast in 100 c.c. of water for 2 hours, then autoclaving for forty to sixty minutes and allowing to settle a week before using.

Of all the abovementioned media, bean extract medium gives best growth. The next best are soil extract and marmite mannite medium. In the first case slant growth in the limits of visibility can be observed after twenty-four hours. A growth to the full extent is reached after about seventy-two hours. In the second case growth is apparent only after thirty-six hours, while full growth is attained after no less than eighty-four hours.

The results with ash maltose medium were not so satisfactory. Better results were obtained on ash medium with sucrose as the carbohydrate. This made us direct our attention to the prominent part which the specific carbohydrate might play in determining and stimulating a favourable

growth. This surmise of ours was justified later on by the fermentation experiments which we carried out at a later stage of our work and which will be described further on.

We also want to remark in this connection that for critical biochemical work, media containing such ingredients as " marmite " and wood ash are not so easily controllable as those containing more definite and isolated compounds. In fact the objection could be raised as well in the case of Yeast extract.

* * *

(A). Two kinds of colonies were found in the plates, specially in Bean extract medium, on which the emulsion from the nodules of *Cajanus indicus* was plated. One type of colonies, as mentioned before, showed abundant gum so that when an attempt was made to take out a little bit of it with the loop regular long threads were formed. The other type of colonies did not show so much gum. Both of them however correspond to the general description of nodule-bacteria, and so special attention was devoted to them. The gum formation is by no means a character on the basis of which an identity can be accepted or rejected. We preserved carefully the stock of both kinds of colonies, namely the one producing greater and the other producing a lesser amount of mucilage; and in subsequent experiments the " concomitant " was tested along with the others.

We tried this latter organism by different methods and we found that, although the organism behaved in many ways like *Rhizobium radicicola*, it failed to produce nodules on *Cajanus indicus*.

A fuller description of this interesting organism is published elsewhere. We have proposed for it the name of *Bacillus concomitans*.

* * *

The second organism isolated from *C. indicus* was the typical *Rhizobium* and once identified with the preliminary tests, it was set apart for further studies together with the organisms isolated from the two other host plants singled out for our research.

The colonies of the organism of *C. indicus* were round, smooth, watery, entire, translucent, convex.

Streaks are raised translucent, spreading over the whole surface and often flowing down and accumulating to some extent at the bottom.

When forty-eight hours growth smears were prepared a Gram negative straight rod with round ends was revealed. The rods measured from 1·5 to 3·0 microns. Branched forms were never noted in this organism either.

(B) *Isolation of organisms from the root nodules of* D. biflorus *and* Ps. tetragonolobus.—The nodule organisms from *D. biflorus* and *Ps. tetragonolobus* were isolated from the root nodules of the plants grown in our laboratory in the same way as the organism from *C. indicus*. In their isolation no contamination was met with.

After isolation, the organisms were tested for their identity both by their reactions on different media and by the biological test. They were found to be true to type.

The colonies of *D. biflorus* were more watery, transparent, becoming somewhat milky as age advances, not very slimy, glistening, smooth, entire, not as convex as those of *C. indicus* (flattening out with age), and with a tendency to coalesce and extending up to 8 mm. in diameter.

Forty-eight hours mounts from marmite mannite agar showed Gram negative rods varying from more or less cocci-bacilli form to well-shaped rods up to 2·5 microns in length. Swollen vacuolated forms were found when mounts were prepared from old cultures.

Colonies of *Ps. tetragonolobus* organisms were found to be much smaller compared with those of *C. indicus* and *D. biflorus*. They were drop-like, more convex, not very mucilaginous, glistening, opaque, smooth, entire, extending up to 2–3 mm. in diameter.

Smears showed Gram negative rods with round ends. Vacuolated forms were very often seen. Branched forms were not seen, neither in artificial cultures nor in nodules.

Chapter II.

Physiological and Biochemical Reactions in Experimental Media.

If soil inoculation with a given nodule bacterium is to be done, with a view to grow some particular leguminous plant that has not hitherto been grown in a particular area, it is quite essential that the identity and specific affinities of the micro-organisms be established or at least the probabilities of cross-inoculation, when a specific organism is not available, or other leguminous crops have previously been grown there. On the other hand, in order to divide the organisms into various groups and assign them definite reactions towards various media, it is necessary to examine as many of the organisms in that group as possible, if any generalisation is to be attempted.

With regard to the organisms isolated by us very meagre data were at our disposal, so it was thought necessary to make a fresh and systematic study of the cultural reactions and physiological behaviour in general.

Reaction on potato medium.—As already mentioned, Löhnis and Hansen (1921) have proved that Rhizobia organisms do not grow, or grow very scantily on potato slants. So potato slants were prepared by cleaning the potatoes thoroughly and cutting out of them, by means of a cork-borer, cylinders just wide enough to get into test-tubes. These cylinders were cut diagonally so as to give slant surfaces. They were then washed in water and placed into a solution of sodium carbonate (1 in 500) for half an hour. They were then taken out and washed several times in water and transferred to sterilised test-tubes having small pieces of glass rods as supports and a few c.c. of water at the bottom, and were autoclaved for half an hour. In order to avoid overcooking the tubes were placed in the autoclave after the steam had begun to pass out freely.

After a few days the slants prepared as above were inoculated with the organisms in question. The result was that except in the case of *Ps. tetragonolobus* no growth was observed, while in *Ps. tetragonolobus* sometimes very slight growth was noticed.

Carrot slants were also prepared in the same way as potato ones and inoculated with our organisms. No growth was observed in any case.

Milk medium.—Löhnis and Hansen (1921) showed that considering the growth of Rhizobia on milk media they can be divided into two definite groups. Members of one group form a " serum zone " and they correspond to those which are fast growers, whilst those of the other group do not produce " serum zone " and belong to the slow growers. Cowpea (*Vigna catiang*) group organisms are classed as belonging to the latter one. In 1925 Stevens introduced indicators in the milk and was able thereby to note the changes distinctly. He divided the nodule bacteria from their reaction on milk into three groups. (1) Those that produce acidity in milk. (2) Those that produce alkalinity in milk but at the same time give a 'serum zone'. (3) Those which do not produce 'serum zone' but are basic in reaction. According to this classification the Cowpea group organisms are placed in the third group. In testing our micro-organisms we observed the unexpected facts that *C. indicus* and *D. biflorus* commonly placed in the Cowpea group do not behave in this reaction as the members of the third group.

Milk media, both containing indicators and no indicators, were prepared in the following way :—

Fresh pasteurized milk was obtained and placed in a shallow dish in the refrigerator overnight. Next morning the skimmed milk was siphoned out into another vessel leaving the cream in the first one. It was tested to see whether it titrated above—1·7% normal (Fuller's scale). In most

cases there was no need to adjust to normality. It was tubed in sterile tubes, plugged and sterilized in the water-bath on four successive days for twenty minutes, care being taken that the tubes were placed after the water had begun boiling.

When milk containing indicators was required, they were added before tubing. In the case of litmus milk 2% of a standard solution of Kahlbaum's azolitmin was added. Also brom-thymol-blue milk was prepared on several occasions. All these were inoculated with the organisms in question.

Their reaction in the beginning was alkaline in all cases. But *C. indicus* organism reduced the litmus and later on produced acidity. While the "concomitant" from *Cajanus indicus* and the organism from *D. biflorus*, and Goa-bean produced 'serum zone' and remained alkaline throughout. (See Plate V, Fig. 1).

From this it appears, as hinted above, that if the *C. indicus* and *D. biflorus* organisms are to be retained in the Cowpea group and the paramount criterion is the biological test (cross-inoculation) then the aggrupation of organisms obtained by the milk reactions is not substantiated.

In our attempt to prepare milk medium we tried to autoclave the milk, containing indicator or not, by placing the tubes in the autoclave after the steam had begun to come out, allowing then the temperature to rise to 110° C., stopping the gas and when the pressure went down finally transferring the tubes to the refrigerator. It was found that this method of preparing the milk served as well as the ordinary process of sterilising successively for four days. It saved time in addition.

Glycerol phosphate medium.—Glycerol phosphate medium having the formula given below was tried to see what changes in morphology occurred as Percival suggests that bacteriods are formed in this medium. After several attempts it was found that none of the abovementioned stocks would grow. A modification, however, is contemplated.

Distilled water	.. ..	1000·0	c.c.
Magnesium sulphate	.. ..	0·1	gm.
Di-potassium phosphate	.. ..	2·0	,,
Glycerol	.. ..	10·0	,,

heated to boiling, filtered, tubed, sterilised for three successive days.

Carrot agar.—Carrot agar medium was prepared according to Müller and Stapp (1925). 250 gms. of well washed and finely minced carrot were cooked in the steamer with 500 c.c. of water for half an hour. The filtrate was taken out and made up to 500 c.c. and adjusted to pH 7·0 with a strong

solution of sodium carbonate. 500 c.c. of 3·6% neutral water solution of agar was added to the extract, mixed well, tubed and sterilised at 110° C.

Slants of the above media, when inoculated with all our stocks, gave a fair growth but they became dry much sooner than in the case of other media. They turned the medium brown suggesting tyrosinase reaction. So we prepared a medium to which a given amount of tyrosin was added and the reaction verified.

Tyrosinase reaction.—It was of interest to test in our Indian micro-organisms the presence of an enzyme acting on tyrosin.

After studying the formula used by Stapp, 1923 and used by Almon, we selected Thornton (1922), mannitol nitrate-asparagin medium to which 0·15% of tyrosin was added. It was found that all our cultures produced browning in the medium, but they differed in their grades of darkening. *Ps. tetragonolobus* organism produced the darkest colour and *Cajanus indicus* organism the faintest.

Yeast water mannitol agar is not suitable for this purpose. As has been indicated by Eckhardt, etc. (1931), the natural colour of the yeast water substrate interferes with the colour changes of the tyrosinase reaction.

The formula of tyrosin mannitol nitrates asparagin medium used in our experiments is as follows :—

Water	1000·0	c.c.
Mannitol	1·0	gm.
Asparagin	0·5	,,
Potassium nitrate	0·5	,,
Di-potassium phosphate	1·0	,,
Magnesium sulphate	0·2	,,
Calcium chloride	0·1	,,
Ferric chloride	0·002	,,
Sodium chloride	0·1	,,
Agar	25·0	,,

The presence of gelatinase.—Burill and Hansen, 1917, proved that the nodule organisms are capable of slowly liquefying gelatin, and it was further testified by Stapp (1924) that almost all the nodule organisms belonging to every cross-inoculation group liquefy gelatin slowly. Others dissent radically from this view. Müller and Stapp (1925) emphasised the importance of using only gelatin of the proper reaction. Eckhardt and others (1931) pointed out that the use of Witte's peptone favoured the growth. This was proved to be correct also by our experiments. The kind of gelatin and the kind

of peptone do have a great effect on the growth of organism in gelatin and in its liquefaction.

We carried the final experiments in the following way.

Beef extract peptone gelatin was used according to the following formula :—

Beef extract	..	..	3 gm.
Peptone (Witte's)	..	..	5 ,,
Distilled water	..	..	1000 c.c.
Gelatin	..	..	20–25 %

Adjusted to neutrality.

Contrary to the finding of Müller and Stapp (1925) better results were obtained with Witte's peptone.

In our experiments it was noted that if instead of gelatin stab, gelatin slants were used, liquefaction when it occurs takes place much earlier. The reason for a better growth in gelatin slants than in gelatin stabs may be that in the former a much better oxygen supply is available. Our organisms are strict aerobes, though probably in varying degrees. The reaction of these organisms towards gelatin *plus* oxygen can be made clearer by comparing the normal results with those obtained by the shake culture method.

The result of our observation is that the concomitant bacillus does not seem to liquefy gelatin, while Goa-bean organism liquefies gelatin sooner than the *C. indicus* and *D. biflorus*. They all grow meagrely and that after a long time and get somewhat dirty coloured, indicating tyrosinase reaction. We therefore consider that by our choice of the slant method we can observe gelatin liquefaction in our bacteria and are inclined to give a more favourable report on the subject of liquefaction than that given by Walker. A comparative study of other strains with our slant method is contemplated.

With our simple slant method complete liquefaction takes place in about a fortnight, while the method reported by other authors requires months (*cf.* for instance Eckhardt, Baldwin and Fred, 1931). In our laboratory the stab method gave liquefaction after one month and slant within a fortnight.

It is to be noted that the amount of growth and the liquefaction are not interdependent. In our cultures we obtained growth at the third or fourth day, and beginning of liquefaction after ten days as a rule. But in any case we have to point out that gelatin is not the ideal medium for the growth of our organisms and even in case of good liquefaction the growth may be called slow and in addition scanty.

Dye bacteriostasis.—Stevens (1925), Wright (1925) and various other authors have studied the relation of the growth of nodule organism to crystal violet and have shown that various groups will tolerate different concentrations of the reagent. In our experiments we tried two kinds of media, solid and liquid, and curiously enough with different results. The liquid medium is that suggested by Stevens. In our experiments we used a sucrose peptone solution containing 2·0 gms. of monopotassium phosphate, 0·1 gm. of magnesium sulphate, 20·0 gms. of sucrose, 10 gms. of peptone per litre, sterilising on three successive days. It was noted that Goa-bean organism grows better ; *D. biflorus* organism grows, if at all, very little ; while the *Cajanus* organism and its concomitant did not grow at all. On the contrary all the organisms grew quite well in Leonard's solid medium (1931) containing crystal violet in 1 : 80,000 concentration and produced colonies evenly coloured.

The formula of Leonard's medium is :—

Soil extract	.. ..	1000·0 c.c.
Di-potassium-phosphate	.. ..	1·0 gm.
Sodium nitrate	.. ..	1·0 ,,
Glycerol	.. ..	10·0 ,,
Agar	.. ..	10–15 ,,

(but we used 20 gms. of agar for the reasons mentioned at the beginning of the first chapter).

Soil extract was prepared by heating 1000 gms. of garden soil with one litre of tap water in the autoclave for thirty minutes. A small amount of calcium carbonate was added and the whole filtered through a double filter paper till the filtrate came through it quite clear.

The phenomenon has also been observed in the case of Congo-red.

We use the following formula suggested by Fred, Baldwin and McCoy (1932) :

Mannitol	.. ..	10·0 gm.
Potassium nitrate	.. ..	0·5 ,,
Di-potassium phosphate	.. ..	0·5 ,,
Magnesium sulphate	..	0·2 ,,
Sodium chloride	.. ..	0·2 ,,
Ferric chloride	.. ..	Trace.
Manganese sulphate	.. ..	Trace.
Water	.. ..	1000·0 c.c.
Agar	.. ..	25·0 gms.

Congo-red was added just before tubing 1 in 400 aqueous solution of the dye.

All the cultures of our strains produced whitish colonies without the absorption of the dye. This is an important test which differentiates our organisms from *B. tumefacians* which absorbs the dye.

We also found this medium preferable to others as the growth upon it is not too abundant and the colour absorption reactions are more delicate and distinct.

Hydrogen-ion reactions.—Fred and Davenport (1918) worked extensively on the reactions of Hydrogen-ion concentrations and limits of growth of the different cross-inoculation groups. We proceeded to study our organisms in this respect and tried to determine the range of pH of the cultures isolated by us. For this purpose Bean extract medium was selected as our previous work had shown it to be the most favourable medium for their growth.[1]

Slants were prepared varying from pH 3·0 to 9·6 and inoculated with our strains. It was found that *B. concomitant, C. indicus* and Goa-bean organisms had their lowest limit 4·8 and their highest limit more than 9·6 (above the limits of the apparatus), while *D. biflorus* organism had 5·2 as its lowest limit and the highest, as the others, more than 9·6. All showed their optimum growth at 6·6 to 7·8 pH.[2]

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[1] In the beginning, we prepared media tubed and autoclaved them and then adjusted the pH with normal acid or alkali using sterile pipettes and other apparatus needed. But once we found that the control also was contaminated perhaps by chance. In our procedure, everything that was used was sterile and manipulations were done aseptically excepting the acid or the alkali used. Perhaps the contamination was pure chance. Strange as it looked, even to ourselves, we decided in our later attempts to sterilize the acid and alkali as well before using them. McClung (1932), we have found later, used also sterile reagents. The fact is that never since did we experience contamination.

[2] In this connection, we may mention that it occurred to us that the acidity of the juice of tamarind plant, to mention a common Indian plant, might be in the way of the symbiotic micro-organism and prevent the connection required for nodule formation. To investigate this point, we grew some tamarind plants in the ordinary garden soil previously used for the growth of nodules of *Cajanus, Dolichos* and Goa-bean. When the seedlings were about three weeks old, they were removed carefully, without disturbing their roots very much. The soil sticking to the roots was collected and its pH was determined, the pH of the sap of the root was also taken. The result was that the pH of the soil, before sowing the seeds, was 6.5 and that of the soil sticking to the roots was 5.2 and the extract of the root has 6.3. It thus appears that the acidity of the soil surrounding the rootlets does not increase so as to prevent the growth of the nodule organism (minimum pH 4.8). It was considered that the sap may contain some substance which would prevent the attack of the organism. Consequently, a media was prepared out of the extract of the rootlets of *Tamarindus indicus*, and adjusted to 6.3, its original pH, and inoculated with the organisms. It was found that the four strains organisms grew quite well. It is yet to be investigated whether a particular organism would adapt itself to form nodules on *Tamarindus indicus* roots, or, perhaps, its rootlets secrete a particular substance which prevents the action of these bacteria.

For the adjustment the colorimetric method was used by means of Hellige Comparator on the following scale of indicators : Bromphenol blue 3–4·6, Methyl red 4·4–6·0, Bromthymol blue 6·0–7·6, Cresol red 7·2–8·8, Thymol blue 8·0–9·6, Thymol blue 1·2–2·8, Cresol purple 5·2–6·6, Phenol red 6·8–8·4, as recommended by Clark and Lubs.

Fermentation and utilization of various carbohydrates.—It is reported by different authors that the relative value of different carbon compounds varies in the case of the growth of different nodule organisms. Differences in their reaction on sugars have been noted and also their preference for particular sugars. Temple (1916) reported that sucrose and glucose give better growth than lactose. Joshi (1920) found out, working with the organisms of Cowpea, pea, sunnhemp, etc., that mannitol stands best for the growth of the nodule organisms. Müller and Stapp (1925) showed that the organisms belonging to different cross-inoculation groups behave differently towards carbon compounds. McCoy, Baldwin and Fred (1931) have studied the growth and fermentation characteristics of *Rhizobium lupini* on a nitrate medium containing different carbohydrates and have found that there was an obvious disparity of results when different sugars were taken. Besides the quantitative growth, the fermentation reactions are of great interest. The organisms belonging to various cross-inoculation groups have been studied by Fred and Baldwin (1927), who observed that the organisms of Cowpea, Soy-bean and Lupine produce alkalinity in nearly all the sugars tested by them, while those of Alfalfa, Pea, Bean, and Red Clover show mostly acidic reaction. However, variations in their reactions were observed in the case of different strains of the same organism. It is accepted as a common rule that the organisms belonging to the fast-growing group produce acidity, especially in galactose, and this is considered to be one of the best criteria of differentiating the organisms of the two groups.

It was our point to enquire into the constancy of these findings in the case of the organisms under research.

In our experiments we used the medium recommended by Baldwin and Fred (1927) having the following formula :—

Agar	.. ..	25·0 gm.
Di-potassium phosphate	.. ..	0·5 ,,
Potassium nitrate	.. ..	0·5 ,,
Magnesium sulphate	.. ..	0·2 ,,
Sodium chloride	.. ..	0·2 ,,
Calcium carbonate	.. ..	3·0 ,,
Sugar or any organic carbon compound	..	10·0 ,,
Distilled water	.. ..	1000·0 c.c.

5 c.c. of 0·5% alcoholic solution of brom-thymol-blue added per litre to indicate the changes in the acidity.

As usual to avoid hydrolysation of the sugars these were added last in the preparation of the medium. Tubes were placed in the autoclave in small batches after the steam began to flow freely. They were sterilised at one atmosphere pressure and rapidly cooled by transferring them to the refrigerator as soon as the pressure went down. Sugars belonging to various series, *i.e.*, pentoses, hexoses, disaccharides and trisaccharides and mannitol, were used and the growth of the organisms on them and their reaction were studied. The results of these studies are given in Tables 1 and 2.

TABLE 1.

Growth of the Organisms on a Nitrate Medium containing various Carbon Compounds.

Sugars	*Cajanus indicus* organism	*Dolichos biflorus* organism	*Psophocarpus tetragonolobus* organism	*C. indicus* concomitant organism
Rhamnose ..	++	++	++	++
Arabinose ..	++	++	++	++
Xylose ..	++	++	+	++
Fructose ..	++	++	++	++
Glucose ..	++	++	++	+++
Galactose ..	++++	++++	++	+++
Mannose ..	+++	++	+	++
Sucrose ..	++++	++++	++++	++++
Lactose ..	+++	++	+	++
Maltose ..	+++	+++	++	+++
Raffinose ..	++++	+++	+++	+++
Dextrin ..	+	+	+	+
Mannitol ..	++++	+++	+++	++++

+ Scanty growth ; ++ Moderate growth ; +++ Good growth ; ++++ Profuse growth.

TABLE 2.

Fermentation Characteristics of the Organisms on a Nitrate medium containing various Carbon Compounds.

Sugars	*Cajanus indicus* organism	*Dolichos biflorus* organism	*Psophocarpus tetragonolobus* organism	*C. indicus* concomitant organism
Rhamnose ..	−−	++	++	−−
Arabinose ..	−−	++	++	−−−
Xylose ..	−−	+++	+	−
Fructose ..	+	+++	++	−
Glucose ..	−−	+++	+	−−−
Galactose ..	−−−	+++	+++	−−−
Mannose ..	−−	++	++	−−
Sucrose ..	++	+++	+++	−−
Lactose ..	−−	++	++	+
Maltose ..	++	+++	+++	+
Raffinose ..	++	+++	++	+
Dextrin ..	+++	+++	+++	+++
Mannitol ..	−−	++	++	..

+ Slightly alkaline ; ++ Moderately alkaline ; +++ Strongly alkaline.
− Slightly acid ; −− Moderately acid ; −−− Strongly acid.

From the examinations of the tables it will be clear that sucrose and mannitol give best growth. Raffinose stands next. Poor growth was observed on pentoses and dextrin.

From the fermentation characters it is clear that the *D. biflorus* and *Ps. tetragonolobus* organisms produce alkaline change in all the carbohydrate compounds used, agreeing in this respect with the slow-growers, while *Cajanus indicus* organisms and its concomitant both have acid and alkaline reaction depending on the sugars. Judging from their reaction on galactose they behave as fast-growers. It is interesting to note that all the organisms at the start show an alkaline reaction in all the sugars,

while in the end the reactions are as indicated in the tables. The results of two weeks' observations are reported in these tables.

Chapter III.

To establish conclusively the identity of the organism by the biological test we decided to grow plants in flasks and test-tubes containing medium as advocated by Garman and Didlake. This is very handy and convenient method of growing plants in sterile conditions. It does not require too much attention to keep the experiment going and maintain sterile conditions. (See Plate VI.)

For this purpose we first selected the medium for growing seedlings, recommended by Thornton, having the following formula :—

Di-potassium phosphate	.. ..	0·5 gm.
Magnesium sulphate	.. ..	0·2 ,,
Sodium chloride	.. ..	0·1 ,,
Calcium phosphates	.. ..	2·0 ,,
Ferric chloride	.. ..	0·01 ,,
Agar	.. ..	10·0 ,,
Water	.. ..	1 litre.

This medium was put in 50 c.c. and 150 c.c. amounts respectively into big Pyrex test-tubes of 100 c.c. and Erlenmeyer flasks of 250 c.c. They were plugged and sterilised at one atmosphere pressure. Seeds of *Cajanus indicus*, *Dolichos biflorus* and Goa-bean, Lucerne, Cowpea and Soy-bean were sterilised in the same way as the nodules by subjecting them to the action of 0·2% mercuric chloride solution and exhausting the air to insure complete contact of the disinfecting solution with the whole surface of the seeds. Fifty seeds of each of the two plants *C. indicus* and *D. biflorus* were put in a tube ($1\frac{3}{4}$" by 7") and washed together. In the case of *Ps. tetragonolobus* on account of the size we used about 15 (fifteen) seeds.

Sterilisation of seeds was continued (3 to 5 minutes) for a longer time than in the case of nodules, as it was found that they were not injured in any way by this prolonged action. This was manifested by the fact that all the seeds germinated and produced fair growth as was also proved by Fellers (1919). After sterilisation of the seeds they were washed in several (at least six) changes of sterile water. The last wash as suggested by us in the case of nodule washing was plated out to make sure of the sterile condition of the seeds. They were embedded aseptically in the surface of the agar, two seeds in the case of every test-tube and four seeds in every flask, taking care that the seeds were placed near the walls so that the growth of the roots and the formation of nodules could be conveniently seen. As

soon as the seeds germinated, they were inoculated with a loopful of the culture to be tested by spreading the inoculum near the seedlings, leaving some flasks with their seedlings alone as control. The seeds were not inoculated with the organisms before sowing, as is a common practice, paying attention to the remark of Hiltner and Stormer (1930 *a*) that direct inoculation of large seeds may not lead to nodule formation, or to the contention of Haas and Fred (1919) that the mercuric bichloride remaining in the seed coat has an injurious effect on bacteria, although this is not likely to occur in our method. They were allowed to grow till they wilted. Growth was quite good in all cases. After a period of three weeks from their germination a few plants were taken every week and examined for nodules. In all cases, whether inoculated with the organisms or not, we found that no nodules had been formed.

This experiment was repeated several times with the above-mentioned medium but we always obtained negative results. It was thought proper to try some other medium and repeat the experiment. Ashby's modified medium recommended by Fellers (1919) was chosen having the formula given below :—

Mannite	.. ..	10·0 gm.
Monopotassium phosphate	.. ..	0·2 ,,
Magnesium sulphate	.. ..	0·2 ,,
Calcium sulphate	.. ..	0·1 ,,
Calcium carbonate	.. ..	1·0 ,,
Distilled water	.. ..	1000·0 c.c.
Agar	.. ..	10·0 gm.

In this case too, as before, negative results were obtained. Then we proceeded to use Bean extract medium as we had found all along our work that it gave the best growth for nodule bacteria. Again we obtained negative results.

In our above-mentioned experiments we had the flask and tubes exposed to the light. Although we have sufficient evidence to be rather sceptical about the destructive action of light on nodule bacteria, we thought to exclude the possibility of that objection being raised against our experiments. Consequently another experiment was started with the above-mentioned three media and the flasks covered with black papers. This time too we found the plants and the roots quite well developed but no nodules were formed on the inoculated plants.

At this stage we are not in a position to say why the method of Garman and Didlake, 1914, which is so handy and convenient is not successful in

India. Joshi (1920) also seems to have tried the method and abandoned it. He writes, "for cross-inoculation the plants which are to be inoculated may be grown in (1) nutrient solution contained in flasks or test-tubes, (2) in agar contained in flasks or in test-tubes, which is advocated by Garman, or (3) in sand in pots.

" The first two methods though convenient were not found very successful in this laboratory on a previous occasion and were therefore thought unsuitable for cross-inoculation."

We did not continue our work in this direction, as the method was not absolutely necessary for the study of the physiological reactions of the organisms. We visualise the possibility of adjusting the conditions so as to make this method of culturing plants and inoculating them with nodule organisms a successful procedure. It is surmised that by changing the conditions of the procedure and perhaps also the condition of physical texture of the medium, this method can be made successful. We hope to devote in future some attention to this work.

The other method of growing the plants in sterile soil or sand was adopted. We attempted these experiments with ordinary flower-pots.

The flower-pots, of about eight inches diameter, were first sterilised in the autoclave at 120°C. for an hour on two consecutive days. The soil was first charred and then the pots were filled with it and autoclaved for one hour in order to accomplish complete sterilisation as mentioned by Brown and Stallings (1921). Seeds were sterilised by the method mentioned before. They were then transferred to the pots with sterile forceps and sown into soil and watered with ordinary tap water. At the close of the experiment we found the results were not consistent. So we changed the procedure. The chances are that soil might not be properly sterilised. It is very difficult to sterilise properly the ordinary soil even if the process is carried out for hours on end. We also adopted sterile water for watering the plants. The water coming from our Stoke's still (1933 model) is found to be quite suitable.

The second set of experiments were arranged as suggested by Koch and Butler (1918). Earthen pots were filled with burnt and sterilised sand to which the following inorganic salts were added: 12·5 gm. of calcium carbonate, 10·0 gm. calcium phosphate, 5·0 gm. potassium sulphate and 1·3 gm. magnesium sulphate per 25 kilogrammes of sand. The pots were saturated with water, covered with paper and sterilised in the autoclave for two hours at one atmosphere pressure on two successive days.

After sterilisation the sterilised seeds were sown and watered with sterile water. When the seedlings began to appear the pots were inoculated

by means of a sterile pipette with the organisms to be tested, using a heavy emulsion of them. It was found that plants died in about three weeks before we could expect nodule production.

As the method of Koch and Butler does not seem to be without objection and the inconsistency of results may seriously upset the plans of delicate experiments we decided to proceed independently and devise a method of growing the plants without fear of contaminations which could be relied upon to work under our conditions and with our Indian plants. An important point here is not only to set the experiment going under sterile conditions but to maintain these conditions all through without undue complication either in the apparatus or in the manipulation. Our procedure was as follows :—

The sand was washed with ordinary tap water several times till it showed very slight indication of presence of nitrate (Ring test). It was then heated in flat metal dishes ("lagan") in the hot oven for twenty-four hours continuously at 120°C. to eliminate any nitrogen nutrient compounds and at the same time to sterilise it as thoroughly as possible. The sand was well stirred every few hours so that the heat would reach every portion of it. The pots were sterilised at 120°C. for half an hour on two days. They were then filled with burnt sand. Four small portions of glass tubes open at both ends, having a bore wide enough to let the seeds pass through were fixed in the sand in each pot at equal distance from the centre. A big mouthed test-tube with the bottom cut was thrust into the centre of the pots. All these were plugged with cotton wool. Before fixing the tube in the sand the pot was covered with two or three folds of cotton cloth which was tied round under the rim of the pot with a string. This cotton cloth was perforated just enough to allow the tubes to protrude. If the holes happen by accident to leave too much space around the protruding tube the gap may be plugged with cotton wool. The whole pot so prepared was sterilised at 120°C. for half an hour.

The seeds sterilised by the method described before were placed into the sand through the small tubes under aseptic conditions. They were then watered one by one with sterile water through the small tubes and these plugged again. Watering with sterile water through the individual tube was continued for about five to seven days till the seeds germinated. The watering was then effected through the special big tube placed in the centre. They were then inoculated with a heavy emulsion of the organisms to be tested. When the seedlings grew long enough to come out of the tube, the plugs were removed, the seedlings were allowed to come out freely and sterile cotton was wrapped round their stems. All these manipulations

were done as much as possible under aseptic conditions (see Plate VII, Nos. 5 and 6). After inoculation the plants were also watered with sterile water through the test-tube fixed in the centre of the pot, plugging the tube every time after watering. Plants were supplied every week through the watering tube with Crone's modified solution as used by Bryon having the formula as follows :—

Potassium chloride	.. ..	100·0 gm.
Calcium sulphate	.. ..	25·0 ,,
Calcium phosphate	.. ..	25·0 ,,
Iron phosphate	.. ..	25·0 ,,

For the solution one and half grammes of this mixture were added to one litre of distilled water. In addition to this 0·75 gm. of sodium carbonate was added to it. The mixture was well shaken and allowed to settle overnight, then it was filtered and autoclaved before using.

Plants were allowed to grow in this way and then gradually removed a few every other week to examine nodule formation. By this method we got quite consistently successful results. Consequently in all the experiments that followed we adopted this procedure and it is satisfactory to mention that we never met with any complication whatsoever.

In this manner we first tried to test the identity of the organism isolated from *Cajanus indicus*, *Dolichos biflorus* and *Psophocarpus tetragonolobus* by growing the seeds of the plants in sterile pots by the method explained above and inoculating them with their own respective cultures. (See Plates VII–X.)

It was found that the concomitant, isolated from the root nodules of *Cajanus indicus* was not able to produce nodules on the roots of the same plant, *i.e.*, *Cajanus indicus*, grown in sterile conditions and inoculated with the organism in question. This experiment was repeated many times with the same negative result.

Hence we tried to inoculate the same organism into the sand in which *Dolichos biflorus* and Goa-bean seeds were sown in sterile conditions. In this experiment we also got negative results. Whilst the other organism isolated from the root nodules of *Cajanus indicus*, which did not possess so much mucilage, when inoculated into the sand in which plants of *Cajanus indicus* were grown, did produce nodules on the roots, the control showing negative results. Similarly the organisms isolated from *D. biflorus* root nodules and Goa-bean root nodules could produce nodules on the respective plants proving the identity of both the organisms. This experiment was counter-proved several times.

Another experiment on the same lines as above was started to find out the probabilities of trans-inoculation in these organisms. It was noticed that all these organisms could cross-inoculate every other plant. The concomitant as stated before did not produce nodules in any of them. The result is given in Table 3.

A further experiment was done with the true *Cajanus indicus* organism to investigate whether it could produce nodules on the roots of Lucerne, Cowpea and Soy-bean plants grown in sterile conditions. It was noted that *Cajanus indicus* organism could not produce nodules on either Soy-bean or Lucerne plants. It produced nodules on the Cowpea, which is in keeping with the cross-inoculation classification commonly accepted.

TABLE 3.

Results of Trans-Inoculations of Cajanus indicus, Dolichos biflorus *and* Psophocarpus tetragonolobus *Plants with the Four Nodule Bacteria isolated from the Same Three Plants.*

Organisms	*Cajanus indicus*	*Dolichos biflorus*	*Psophocarpus tetragonolobus*
C. indicus ..	+	+	+
D. biflorus ..	+	+	+
Ps. tetragonolobus ..	+	+	+
Concomitant bac. ..	—	—	—

TABLE 4.

Results of Trans-Inoculation of Cajanus indicus *Organism on Cowpea, Lucerne and Soy-bean Plants.*

Organism	Cowpea	Lucerne	Soy-bean
Cajanus indicus ..	+	—	—

Conclusions.

1. The physiological reactions of the three types of Indian nodule bacteria (*C. indicus, D. biflorus* and *Ps. tetragonolobus*) have been studied and ascertained, as is indicated in the tables.

2. In the isolation of the nodule organism from *Cajanus indicus* roots we found with certain characters of constancy a contaminant or rather a concomitant, which has not the characteristics of *B. radiobacter*, the only organism likely to be met with.

3. The *Cajanus indicus* root nodule organism is placed by various authors Löhnis and Leonard, in the Cowpea group, the members of which have got an alkaline reaction on milk medium and also on nearly all sugars. The organism of *C. indicus* isolated by us differs in this respect from the members of the Cowpea group in having acid reaction on litmus milk, and mixed reactions on sugars, acidic and alkaline.

4. The use of gelatin stabs, commonly followed in the study of gelatin reactions,may account for much of the irregularity of reaction and disagreement observed in the conclusions of different authors. Our procedure of the gelatin slants gives clear results, sometimes in half the time, and great advantage will be derived from its general adoption.

5. Garman and Didlake's method proved successful as regards the culturing of host plants, but not as regards the nodule formation. Pending the study of a modification of the same, we devised an easy and reliable method of pot cultures which can be depended upon to give clear results in our exacting conditions of temperature and moisture.

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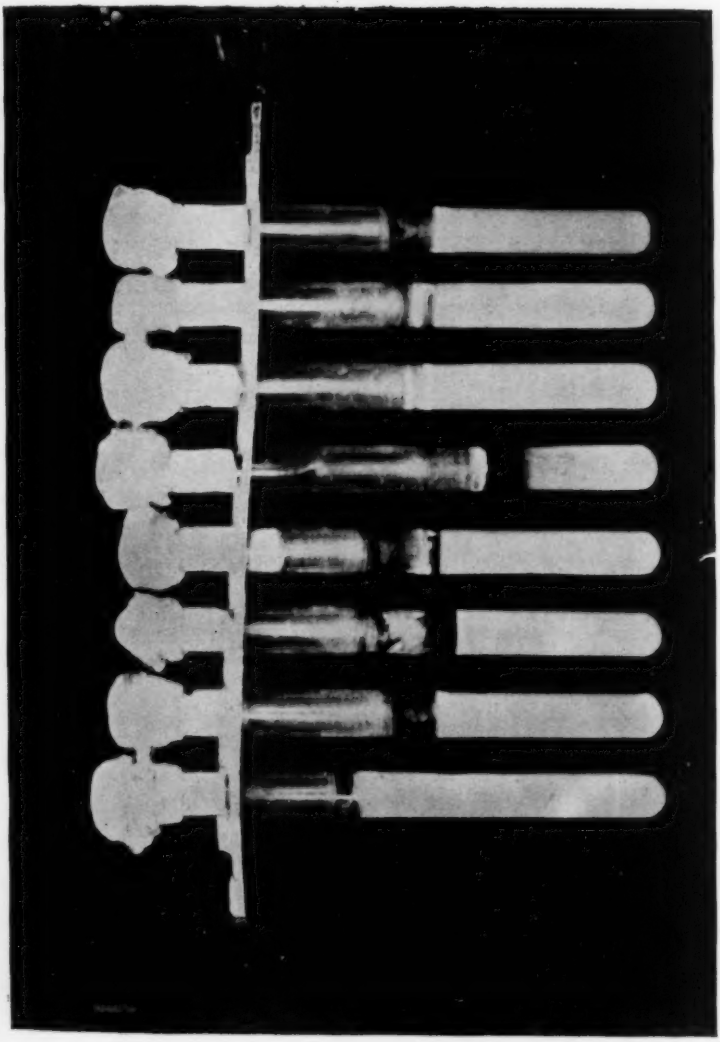

a *b* *c* *d* *e* *f* *g* *h*

FIG. 1.

Litmus milk tubes inoculated with the organisms mentioned below, indicating their reaction.

(*a*) control ; (*b*) soy-bean, alkaline, no serum zone ; (*c*) *B. concomitans*, alkaline and serum zone ; (*d*) Goa-bean, alkaline and serum zone ; (*e*) *D. biflorus*, alkaline and serum zone ; (*f*) *C. indicus*, acidic ; (*g*) Lucerne, acidic ; (*h*) control.

a

FIG. 2.

Garman and Didlake Method of culturing plants in flask containing media.

(We placed the flasks in a metal dish with sand in it in order to keep the flasks in position while shifting and to avoid their clinking together.)

(*a*) Flasks showing the seedling stage of *C. indicus* plants. The plugs are in; the plants have not reached the neck of the flask. When growth has reached a convenient stage, the plugs are eased to allow the plants to pass.

(*b*) *Cajanus indicus* plants showing a stage when nodules can be expected on the roots of inoculated plants.

b

FIG. 3.

a b

FIG. 4.

Method developed in our Laboratory for culturing plants in sand under sterile conditions and for maintaining the same throughout the experiment.

(*a*) and (*c*) (both figures): *C. indicus* plants grown out of the tubes meant for them. In the centre of each pot there is a test tube (with a broken end) through which the plants are watered.

(*b*) Both figures: a pot which shows the first stage of the experiment when seeds are sown in the bottom of the smaller tube.

c a b

FIG. 5

a *b*

FIG. 6.

(*a*) *Psophocarpus tetragonolobus* plants (grown according to the method described in the text) inoculated with its own organisms. They exhibit a better growth than the control (*b*) shown on the right.

a *b*

FIG. 7.

Tuberous roots of *Psophocarpus tetragonolobus* showing differences in growth and presence or absence of nodules. (*a*) Goa-bean roots inoculated with its own organism showing clearly nodule formation and exhibiting much more development than the control; (*b*) less growth in root, stem and leaves; no nodules.

a *b*

FIG. 8.

Tuberous roots of *Psophocarpus tetragonolobus* showing differences in growth and presence or absence of nodules. (*a*) Goa-bean roots inoculated with its own organism showing clearly nodule formation and exhibiting much more development than the control; (*b*) less growth in root, stem and leaves; no nodules.

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A NEW MICRO-ORGANISM ASSOCIATED WITH THE NODULE-BACTERIA IN *CAJANUS INDICUS*.

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In our studies on the Physiology of Indian nodule bacteria, one of the first steps was the isolation in pure culture of the different nodule organisms. After establishing the proper technique, we discovered that from the washed and sterilised root nodules of *Cajanus indicus*, two kinds of bacteria were very often recovered: both possessing the general characteristics of the nodule micro-organisms.

One was classified as the typical *Rhizobium*, the other from the very beginning presented a few peculiarities of its own. When we tried to test this second organism by the ordinary methods of the reaction on potato slants, as suggested by Löhnis and Hansen (1921); on litmus milk as recommended by Stevens (1925), and on Congo-red agar as pointed out by Kellerman (1911), (the detailed procedure is given later on), we found that the organism behaved like *Rhizobium radicicola*.

On the other hand, this " contaminant ", as it was called by us for a long time in the laboratory, produced so much mucilage that when fished out from the medium, regular threads like treacle were formed. This is in marked contrast with the typical nodule organism which produces very little mucilage. As we have said elsewhere, gum formation is by no means a character on the basis of which an identity can be accepted or rejected. But besides this first observable difference, we established a more definitive one, *i.e.*, that it was unable to produce nodules on the plant from which it had been isolated.

We thought that it was just possible that the organism might produce nodules on some other leguminous plants. So another experiment was started with *Dolichos biflorus* and *Psophocarpus tetragonolobus*. Plants were grown under sterile conditions in sterile sand and inoculated with an emulsion of the stock mentioned above. But this time, too, we got the negative result. This experiment was repeated twice to make sure that the organism was

not able to produce the nodules. This sufficiently proved to us that the organism isolated from the root nodules of *Cajanus indicus* having more mucilage was not the normal symbiont, *i.e.*, it does not belong to the *Rhizobium* group. This deduction of ours is further testified by the production of nodules on *Cajanus indicus* plants by the other organism, isolated from their roots (under the same experimental conditions) which does not possess so much mucilage. We, therefore, inferred that the colonies belonged to a contaminant found in the nodules of *Cajanus indicus*. The exact significance of this association, *i.e.*, whether this organism lives at the expense of the *Rhizobium* in an at least partly parasitical way, or whether it is a case of symbiosis which may even increase the nitrogen-fixing activity of the *Rhizobium*, should be the subject of further investigation.

In order to ascertain whether the occurrence of this organism was an accidental though happy finding, or rather a frequent phenomenon, we undertook to repeat the isolation experiments with *Cajanus indicus* nodule organism. We again found the organism under discussion there three out of six times. It must also be noted that in the case of *D. biflorus* and *Ps. tetragonolobus* never was such an association observed.

This fact suggests in the case of *Cajanus indicus* organism certain characters of constancy. The fact of association not only in a saprophytic manner but even in symbiotic relations, at any rate in such a way as to cause jointly the appearance of a given set of symptoms, is by no means rare in microbiology. Under the conditions the name of "concomitant" is more appropriate.

We propose for it the name of *Bacillus concomitans*, nov. sp.

This organism differs from *B. radiobacter* (which has been observed by some workers in such an association) in that it gives negative results in the following tests :—

1. Löhnis and Hansen, 1921, have found that when potato slants are inoculated with a culture of *Bacillus radiobacter* it gives a distinct brown colour, and more vigorous growth takes place, while *Bacillus radicicola* either does not grow at all or at most a meagre, transparent growth may occur. So potato slants were prepared according to the method given in *The Physiology of Indian Nodule Bacteria*, Chapter II. The slants were incubated without inoculation to test their sterility and then inoculated with the concomitant organism and incubated at room temperature even for a fortnight after which time no growth was observed.

2. The use of dyes in media is considered to serve as an excellent differential basis to separate the root nodule organism from *B. radiobacter*. Many

such media have been suggested. But for our purposes we tried the Congo-red synthetic medium of Kellerman (1911), which is considered to be a very selective one ; and also that of Leonard (1931). In the former medium we found the colonies practically not absorbing Congo-red ; whitish colonies were obtained. As regards the second medium the colonies were evenly coloured and not of a big size as is the case with *B. radiobacter*, the colonies of which possess violet or deep blue centres and clear rings.

3. Another medium which differentiates Rhizobia from *B. radiobacter* is milk medium. In plain milk *B. radiobacter* usually produces brown colour and a serum zone is formed. If litmus milk is used litmus is slowly reduced and later on a brown colouration appears. When this test was made it was found that the organism tested did not behave like *B. radiobacter*.

It is again reported that *B. radiobacter* produces an acid reaction when grown on dextrin, whilst none of the nodule bacteria is able to give an acid reaction in dextrin. We got negative results in this test also, *i.e.*, the organism tested produces alkaline reaction.

The colonies of this organism on Bean extract medium were watery, drop-like, mucilaginous, glistening, smooth, entire, convex, varying from 1 mm. to even 5 mm. (Plate XI, Fig. 1).

The streak is raised, translucent, opalescent, glistening with considerable gum (Plate XI, Fig. 2).

The morphology was studied in smear and in hanging drop. When a smear was prepared the organism was found to be a Gram negative rod, with round ends, generally straight, occasionally with slight curvature, varying in size from 1·8 to 3·8 microns. Mounts were prepared from time to time at an interval of twelve hours growth continuing for several weeks, but in no case were branched forms noted (Clark, 1905. Burrill and Hansen, 1917). Various media were also tried and pH altered as recommended by Schönberg (1929), but we failed to get branched forms. We got instead only big rods. Comparative tables of the germination reactions of this organism and other nodule organisms are given in our study of the Physiology of Indian nodule bacteria.

The hanging drop showed a slightly motile rod, with no special tendency for aggrupation and no preference for the edges, under normal conditions.

Various methods were used for flagella staining, Lœfflers, Baily (1930), Gil-Casares, Zetnew and Maneval (1931), Shunk (1921), etc. It was found that Lœfflers' stain always produced very thick precipitate which made the background so obliterated that no good view could be had of the bacteria.

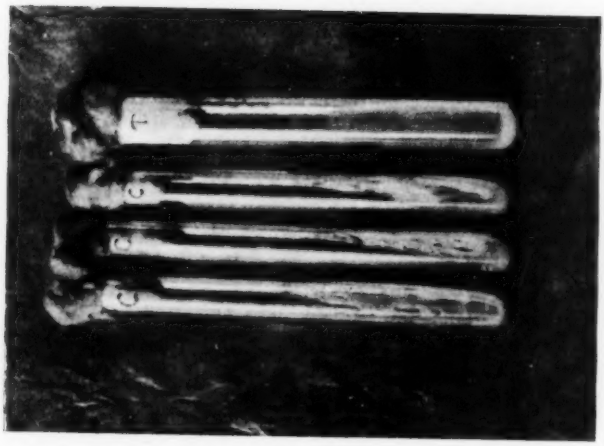

FIG. 2.

Bean extract medium tubes inoculated with *B. concomitans* showing raised, glistening streaks. (T. control.)

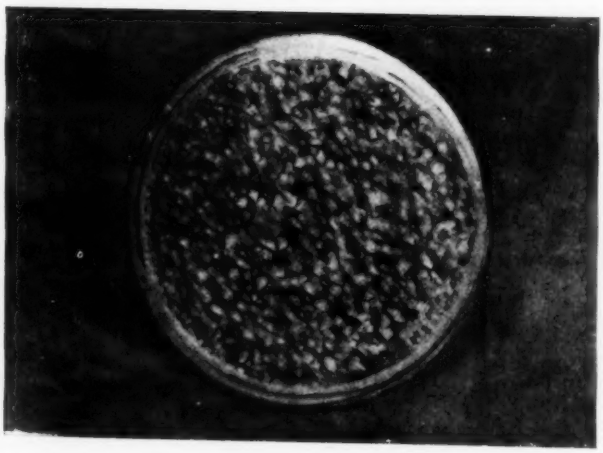

FIG. 1.

Bean extract medium plate exhibiting watery, drop-like, glistening colonies of *Bacillus concomitans*.

Baily's method is acceptable, but did not give us very good results. Zetnew's method of staining gives a very well defined view of the organism, but it is difficult to manipulate. Gil-Casares' which is the routine method in our laboratory was found to give very good results. It is the easiest to handle, gives more consistent results and it is a one-solution stain which also remains in a more stable condition in this country.

The flagella observed in this organism were found to be of the peritrichous type, not very twisted, not numerous, nor very long, as a rule double the length of the rod.

Summary.

A new organism (*Bacillus concomitans* nov. sp.) is described which is found frequently inside the nodules formed in *Cajanus indicus*. When isolated in pure culture it does not produce nodules, it gives a Congo-red negative reaction and possesses many other characteristics which differentiate it from *Rhizobium radicicola* and *B. radiobacter*.

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A NOTE ON THE PETROLOGICAL CLASSIFICATION OF THE BASIC INTRUSIVES OF DANTA STATE (N. GUJRAT).

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AND
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In a short sketch of the geology of Danta State Mr. N. L. Sharma[1] had assigned Post-Aravalli age to all the basic intrusives of Danta State. Petrological study of these rocks shows that there seem to have been at least two phases of basic intrusions between the deposition of the Aravallis and the intrusion of the Erinpura-granite. The older intrusives are more metamorphosed than the younger ones. They have been classified into Epidiorites, Hornblende-schists and Pyroxene-granulites. Epidiorites and hornblende-schists are widely distributed in the State. They are more or less confined to the "middle portion "[2] and are so intimately connected with the Post-Aravalli " Granitoid and Schistose Gneisses "[3] that it is impossible to map them separately on the 1″=1 mile map. Enclaves Enallogenes[4] of hornblende-schists in granitoid gneisses are occasionally seen in the field. Thin veins of epidiorite are sometimes found traversing the sedimentaries of the Aravalli system. Pyroxene-granulites have very limited distribution, but their mode of occurrence is similar to that of the epidiorite.

The second phase of basic intrusion is marked by the Meta-gabbro[5] and Meta-dolerite rocks which occur respectively as intrusive masses and dykes in the granitoid gneisses. These rocks are far less metamorphosed than the older intrusives. Signs of metamorphism are visible in this group of rocks only under the microscope.

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1 N. L. Sharma, "A preliminary note on the Geology of Danta State (N. Gujrat)," *Quart. Journ. Geol. Min. and Met. Society of India*, 1931, 3, No. 1, pp. 17-28.

2 *Ibid.*, p. 18.

3 *Ibid.*, p. 22.

4 S. K. Roy, "Geological and Petrographic Studies in the Hercynian Mountains around Tiefenstein," 1925, p. 55.

5 G. W. Tyrell, *The Principles of Petrology*, 1926, p. 311.

There is yet a third group of igneous intrusives in this area which are absolutely free from all signs of original metamorphism. These are the Olivine-dolerite and Olivine-basalt which are found as dykes or sills traversing the calc-series of the Aravalli system. They are probably Post-Erinpura-Granite in age.

A short petrological description of the above rock-types is given below :—

1. Epidiorites and Hornblende-Schists. (Figs. 1 and 2.)

These rocks occur as intrusive masses, usually associated with the granitoid gneisses. Their main exposures are found in the northern half of the State, comprising the Ambamata, Vav and Ranpur areas. In the Ambamata area, the epidiorite shows a distinct intrusive relation to the Aravalli marble *with the development of garnet and epidote at the contact.* Thin dykes of epidiorite are also seen traversing the Aravalli phyllites in the bed of the Sai river at the north-eastern corner of the State.

Epidiorites are coarse-grained (dioritic) to fine-grained (basaltic) rocks greenish black or black in colour. Some of the medium and fine-grained varieties show well-defined schistose texture and may be termed hornblende-schists. A few of the specimens are calcareous and give effervescence with cold hydrochloric acid. Minute grains of pyrite and copper pyrite are visible in a specimen of epidiorite from the old copper-workings near Ambamata town.

Under microscope, these rocks show usually granoblastic, diablastic or lepidoblastic structures. Relict of ophitic structure (Fig. 1) is distinct in a section of the epidiorite from the Ambamata area. In some of the sections of hornblende-schists, glomero-porphyroblasts of hornblende (Fig. 2) are seen in a lepidoblastic groundmass composed mostly of hornblende and quartz.

In these rocks green hornblende is found to occur as an alteration product of colourless or greenish non-pleochroic diopside. In some of the specimens in which pyroxene has been completely transformed to hornblende but a relict ophitic structure is still retained, there seems to have been a certain amount of absorption of the alkali by hornblende from the felspar laths. Due to this absorption a rim, composed of an aggregate of crystals of deeper greenish blue hornblende, is developed along the margin suggesting a sort of zoning in hornblende (as is apparent from the darker band in hornblende immediately near felspar in Fig. 1). In some specimens of epidiorites hornblende has further suffered a partial alteration to biotite and magnetite.

The felspar in these rocks is plagioclase, varying from oligoclase-andesine to basic andesine in composition. It often shows undulatory extinction.

In some of the slides, felspar is fresh, whereas in others it is much saussuritised. Some epidiorites seem to contain very little felspar and are mostly composed of hornblende, quartz, calcite, epidote, zoisite and chlorite. Occasionally, sphene forms an important accessory. In the calcareous specimens, calcite seems to have been subsequently added being derived from the country rock—the calc-series.

Thus the epidiorites and hornblende-schists are highly metamorphosed basic igneous rocks. In them the pyroxene is usually diopside, and it is wholly or mostly altered to green hornblende. The original structure of the igneous rock is only rarely preserved. Enclaves of hornblende-schists are occasionally seen in the granitoid gneiss; these intrusives are, therefore, assigned to Pre-Granitoid-Gneiss age.

2. *Pyroxene-Granulite and Garnet-Granulite.* (Figs. 3 and 4.)

These rocks possess very limited occurrence. Pyroxene-granulite occurs associated with granitoid gneiss in the northern spur of Rasanio, south of Ambamata town, while veins of this rock are found traversing the calc-gneiss in a hillock near Sadanana, south-east of Danta town. Garnet-granulite occurs as an enclave in Erinpura-granite near Tarsuliaghata, north-north-east of Vav.

Both these rocks are dark-coloured. They are fine-grained in structure and possess massive texture, garnet-granulite being more compact. Under microscope these rocks show granulitic structure.

The pyroxene-granulite of Sadanana is mostly composed of greyish non-pleochroic diopside and labradorite-bytownite (Fig. 3). The felspar is quite fresh but the pyroxene is observed altering into brown hornblende (uralite) at places. A few flakes of biotite are also observed. Magnetite is the only accessory mineral of this rock.

The pyroxene-granulite of Rasanio is also composed of diopside and plagioclase; but the diopside of this rock is green and pleochroic, pleochroism being X=Z=green, and Y=yellowish brown. The alteration of diopside has given rise, probably with an absorption of alkali, to a deep greenish-blue alkali hornblende along the margin. The plagioclase felspar is oligoclase-andesine and is somewhat saussuritised. Sphene is quite abundant, and epidote and calcite are observed as secondary minerals in the rock.

Garnet-granulite is mainly composed of grossularite and labradorite-bytownite (Fig. 4). In this rock no pyroxene is found. Garnet occurs in grains and is altered to epidote and chlorite along its cracks and margin. Sometimes chlorite and epidote are found at the centre of garnet pieces. Sphene and magnetite are the accessory minerals.

As garnet-granulite occurs only as an enclave, nothing definite can be said about its origin. The pyroxene-granulites are basic intrusive rocks in which the original structure has been totally obliterated but there was not much mineralogical change. They, therefore, differ from epidiorites by the fact that in them the pyroxene is either unaltered or is only slightly altered to hornblende.

From their similar occurrence in the field, the pyroxene-granulites and the epidiorites and hornblende-schists seem no doubt to belong to the same phase of intrusion (Post-Aravalli but Pre-Granitoid-Gneiss in age) but the two rock types have most probably been subjected to different grades of metamorphism.

3. *Meta-gabbro and Meta-dolerite.* (Figs. 5 and 6.)

The meta-gabbro forms an isolated hillock (1791 ft. above sea-level) locally known as " Javrashia ", at the southern extremity of the State. It also occurs as an intrusive mass in the granitoid gneisses between Vav and Dhaddarghata pass on the road to Badarmala. Meta-dolerite occurs as a dyke traversing the gneisses in the same locality.

The Javrashia meta-gabbro is a massive rock, greenish-black in colour, varying from coarse to medium-grained in structure. The medium-grained specimens are rather granular, very compact and hard to break. The coarse-grained variety is porphyritic showing big crystals of greenish-black pyroxene, sometimes $\frac{3}{4}''$ long and $\frac{1}{2}''$ wide, embedded in a matrix of felspar with small pyroxene crystals sparingly distributed in it. The pyroxene crystals show schiller effect, but generally their surface is weathered. The Vav-Dhaddarghata meta-gabbro is dark in colour, massive and coarse to fine-grained in structure. The pyroxene is black and shows schillerisation. The meta-dolerite is a dark, fine-grained and basaltic rock.

Under microscope, the Javrashia meta-gabbro shows the usual granitic structure. In other specimens, however, felspar and pyroxene show ophitic structure (Fig. 5). The fine-grained variety of Dhaddarghata area is not only ophitic, but also shows certain amount of effect of pressure by the presence of cataclastic structure of diallage (Fig. 6). The latter has also been partially uralitised.

The meta-gabbro is mainly composed of felspar (usually labradorite) and pyroxene (usually diallage). Some of the pyroxene has altered into pale-green hornblende. The rock is rather poor in opaque minerals like magnetite and ilmenite. Diallage is almost colourless in sections and sometimes shows characteristic double cleavage and parting. It gives biaxial

interference figure with positive sign. Minute opaque or semi-opaque inclusions along the cleavage planes are often observed under high magnification. Usually the alteration of diallage to greenish hornblende begins along the cleavage of the mineral or round its border, leaving the pyroxene still unaltered at the centre. At places, the hornblende crystals show double cleavage.

The diallage of fine-grained meta-gabbro, however, shows a different nature of alteration. It is altered along the border to a brown hornblende (uralite) and it is also much granulated. Occasionally, a few flakes of biotite are enclosed in diallage. The specimen of meta-dolerite shows typical ophitic structure and an abundance of unaltered augite, and not diallage, in the groundmass. Most of the phenocrysts of augite are, however, completely altered to pale-green fibrous hornblende. Magnetite is an important accessory in this rock.

The plagioclase felspar shows both albite and pericline twinning. Occasionally an indistinct zoning is observed in the plagioclase. In some specimens, the felspars show undulatory extinction. The felspar of some of the Javrashia specimens is much altered, the most common alteration product being zoisite, which occurs as big hypidiomorphic crystals inside a granular aggregate of saussurite in which some laths of unaltered felspar are observed.

An interesting dyke of meta-dolerite occurs traversing the crystalline limestone in the bed of the Sai river. It contains lenticular fragments of sheared quartz with some epidote and encrustation of kaolin along the shear planes. These fragments are 3″ or even more in length and look like enclaves. Dr. Coulson[6] has observed similar fragments in the Post-Erinpura dolerite of Sirohee State, and according to him these fragments were caught up by the basic dyke from the Erinpura-granite which was in a semi-solid state at that time.

The rock of the Sai river is, however, much metamorphosed and shows schistose structure. The planes of schistosity of the rock are continuous in the quartz fragments which are consequently much sheared. The Erinpura-granite of the neighbouring area of Harad does not show gneissose structure, whereas the granitoid gneiss at the Danta-Sirohee-Udaipur frontier is distinctly foliated. It thus seems that the quartz fragments in this rock represent probably the enclaves caught up by the basic dyke from a pre-existing pegmatite older than the Erinpura-granite. This pegmatite may

[6] A. L. Coulson, "The Geology of Sirohee State, Rajputana," *Mem. G.S.I.*, 1933, **63**, Part I, 98.

be of the granitoid gneiss phase. Further, this rock is mainly composed of augite, epidote, zoisite with some hornblende, sphene, calcite and cryptocrystalline quartz. It looks more like an epidiorite but differs from it, like the meta-dolerite of Dhaddarghata, in the absence of diopside and an abundance of augite. One or two triangular sections of blue tourmaline are observed in this rock under the microscope. Such tourmaline crystals have also been observed by Dr. Coulson[7] in the metamorphosed basic rocks of Pre-Erinpura-Granite age, at the Sirohee-Udaipur frontier. From these characters, the rock, though highly metamorphosed, is correlated with the meta-gabbro and meta-dolerite phase of basic intrusion. Its Pre-Erinpura-Granite age is further confirmed by the fact that this dyke occurs side by side with those of olivine-dolerite containing titaniferous augite, instead of common augite. The olivine-dolerite is absolutely unmetamorphosed and is regarded as Post-Erinpura-Granite in age. None of the dykes of olivine-dolerite shows the quartz enclaves of the meta-dolerite as mentioned above.

The meta-gabbro and meta-dolerite differs from the epidiorites, hornblende-schists and pyroxene-granulites in their possessing typical structures of the basic intrusive rocks. In addition to this the pyroxene of these rocks is usually a diallage or augite, and not diopside which is the case with the older intrusives ; and further, these minerals are less altered than the pyroxene of the epidiorites. These rocks cannot be called proper gabbros and dolerites as they have undoubtedly undergone some degree of metamorphism, due to which the diallage or augite has been altered partially to hornblende, and also there has been a certain amount of granulation of minerals giving rise to occasional cataclastic structure. Rarely their original structure is completely obliterated, as has been seen in case of the meta-dolerite from the Sai river. These rocks represent an intermediate phase between highly metamorphosed epidiorites and pyroxene-granulites on the one hand, and unmetamorphosed olivine-dolerite and olivine-basalt on the other. A small pegmatite containing tourmaline is found traversing the Javrashia meta-gabbro near its junction with the Erinpura-granite, and the meta-dolerite dyke traverses the granitoid gneiss in the Dhaddarghata area. These basic intrusions have, therefore, been assigned to Post-Granitoid-Gneiss but Pre-Erinpura-Granite age.

4. Olivine-dolerite. (Figs. 7-12.)

This rock occurs as dykes and sills traversing the crystalline limestone, and is best seen in the bed of the Sai river at the north-eastern corner of the State. These dykes and sills are seldom more than 4 ft. in thickness.

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[7] *Ibid.*, p. 48.

In hand specimen, the olivine-dolerite is a dark-coloured massive rock varying from medium to fine-grained in structure. Some of the medium-grained specimens show grains of pyrite or amygdules of calcite. In one specimen, amygdules of calcite and quartz are so abundant that they give a spotted appearance to the rock. The fine-grained variety is basaltic in appearance, but small needle-like laths of white felspar can be easily seen embedded in the dark matrix. Most of the specimens are calcareous and give effervescence with cold hydrochloric acid.

Under microscope, the rock is seen to be essentially composed of titaniferous augite, basic labradorite and some olivine. The augite is pink in colour and is slightly pleochroic from pink to greenish yellow. The felspar occurs as long laths and shows typical ophitic structure with augite (Fig. 7). Sometimes two or even three twinned laths of felspar penetrate and cross each other as if pushing the two halves of a lath asunder (Fig. 8). The augite is idiomorphic in habit and often forms wedge-shaped plates between columnar crystals of felspar. Sometimes the felspar laths enclose between themselves rhombic, hexagonal or even octagonal sections of augite. Occasionally one or two small laths of felspar are seen to be wholly included within an augite crystal. In the fine-grained dolerites, most of the augite occurs as small grains in the ground-mass (Fig. 8).

The alteration of augite is interesting. Some of the titaniferous augite of these rocks is altered into brown biotite and magnetite and also ilmenite. The alteration usually begins along the periphery and cracks; but sometimes the whole crystal is changed, as wedge-shaped sections of biotite enclosed within ophitic felspar are quite common. Further the brown biotite so formed is sometimes seen to change first into green biotite[8] and then into green chlorite with calcite and magnetite.

Pseudomorphs of augite, enclosed between felspar laths and composed of biotite and magnetite with chlorite or calcite (Fig. 9) are very common. Some of these pseudomorphs look like amygdules of calcite bounded by chlorite (Fig. 10). The calcite, however, shows a sinuous margin with chlorite and is often full of dust-like inclusions of magnetite. The form of some of these amygdules is octagonal resembling augite. It seems that the chlorite has been replaced by calcite at a later stage. Felspar laths are sometimes seen enclosing triangular patches of pure calcite (Fig. 11) giving rise to ophitic structure. The felspar laths at the contact with this triangular patch of calcite sometimes show an indistinct zoning, their margins being more altered than the interior. This seems to confirm the view that the

[8] Winchell, N. H. and A. N., *Elements of Optical Mineralogy*, Part II, p. 369.

augite was probably first changed to biotite and then to chlorite which was subsequently replaced by calcite. This change is so characteristic that in some rocks which show, even in hand-specimens, amygdules of calcite and quartz, there is practically no augite left unchanged. In these specimens, it is calcite with dusty magnetite, enclosed within felspar, which gives rise to the ophitic appearance.

The felspar laths usually show carlsbad and albite twinning and sometimes also pericline twinning. In addition to their characteristic cleavage, they are all traversed by traverse cracks. The felspar is partly altered to a crypto-crystalline aggregate of calcite and kaolinite, with occasional flakes of chlorite or micaceous material. At places long needles of zoisite showing characteristic ultra-blue polarisation colours are seen to penetrate into or across the felspar laths.

The olivine is colourless, always altering into pale-green serpentine. It usually occurs in idiomorphic rhombic sections or as short prisms terminated by pyramidal faces (Fig. 12). The sections of olivine are usually included in plagioclase laths. The mineral was, therefore, the first to crystallise from the magma. It is usually altered along the border into magnetite, but sometimes it is completely changed into a mass consisting of magnetite, calcite and quartz, the magnesium being probably removed and replaced by calcium.[9]

This rock differs from the meta-gabbro and the other older intrusives, described before, by the presence of titaniferous augite and olivine, in the peculiar mode of alteration of the pyroxene, and in its almost unmetamorphosed nature. The olivine-dolerite is, therefore, assigned to the youngest phase of intrusions.

5. *Olivine-Basalt.* (Figs. 13-16.)

This rock occurs as very thin veins traversing the crystalline limestone of Sai river and the calc-gneisses of Godda and Danta areas. The veins are sometimes only 2″ in thickness.

In hand-specimen the rock is very fine-grained and massive. It is much darker in colour than the olivine-dolerite. Most of the specimens are calcareous.

The rock-slice of the Sai basalt shows, even with naked eye, numerous sub-parallel and intersecting veinlets or stringers of quite transparent calcite in greyish matrix. Under microscope, the rock shows porphyritic structure and is composed mostly of felspar, magnetite and calcite (Fig. 13). The

[9] *Ibid.*, p. 169.

felspar which is basic labradorite, occurs in rectangular laths which are mostly altered into calcite. Some sections show both albite and carlsbad types of twinning. The interpenetration of two laths of twinned felspar, like that in olivine-dolerite, is also observed. The laths of felspar are embedded in a groundmass of magnetite, altered felspar and calcite. The habit of magnetite is usually acicular, the needles sometimes lying close and parallel to one another as if formed along the cleavage cracks of some mineral. Some of the bigger sections of magnetite appear in the triangular form of the ophitic augite, and some appear hexagonal and rhombic like olivine. Ophitic felspar laths often enclose triangular patches made up of calcite and magnetite. It, therefore, seems that the rock is a highly altered olivine-basalt in which the augite and olivine are completely altered into magnetite or replaced by calcite. The stringers of calcite often cut across the felspar laths showing thereby that the replacement has been subsequent to the formation of felspar phenocrysts.

The specimen of olivine-basalt from the Godda area differs from the above in containing numerous amygdules of calcite (Fig. 14). No stringers of calcite are observed. The rock also contains many hexagonal and rhombic phenocrysts of olivine which have been completely altered into green serpentine, showing ultra-blue polarisation colours. Sometimes the cores of such serpentinised phenocrysts are altered into magnetite. The groundmass is composed mostly of opaque iron minerals, microlites of felspar, and calcite.

The specimen of olivine-basalt from the Danta area differs from the other two, described above, in not having so much calcite. It is, however, very rich in iron. The groundmass is almost opaque consisting mostly of magnetite, leucoxene and microlites of more or less altered felspar. The phenocrysts of felspar and colourless olivine are, however, fresh and unaltered (Figs. 15 and 16). Felspar laths generally show a rough ophitic structure enclosing aggregates of magnetite and leucoxene (Fig. 16). It is thus evident that at least a portion of the opaque iron minerals represents the alteration product of augite. This is further confirmed by the fact that the olivine of this rock is optically positive. If the whole of the magnetite was primary and the magma so rich in iron, then the olivine ought to have been a fayalite which is negative. Similar olivine seems to have been observed by Dr. Coulson[10] in the olivine-basalt, of Post-Erinpura-Granite age, in the Sirohee State. He could not account for the positive character of the olivine in the rock containing

[10] A. L. Coulson, *op. cit.*, p. 96.

"abundant iron and possibly some manganese", for he says, "the mineral may be fayalite though it is positive in optical character."

This rock differs from the olivine-dolerite in possessing no augite at all. The typical structure of a basalt is present, and the groundmass is mostly composed of opaque minerals, like magnetite, ilmenite and leucoxene, with calcite and microlites of felspar. There seems to be a gradation in the alteration of the ferro-magnesian mineral, olivine. In the Danta area to the west, olivine phenocrysts are quite fresh (Fig. 15) ; whereas in the Sai river to the extreme east, olivine is totally altered and replaced by calcite. In the Godda area—lying between the above two areas, an intermediate stage of alteration is observed. Here olivine is present but it is all serpentinized (Fig. 14). It is interesting to note the total absence of augite in this rock, but it appears from the examination of the slides, described above, that the augite was probably in the groundmass but it has been completely altered. The various stages of the alteration and replacement of titaniferous augite finally leading to magnetite, ilmenite and calcite have been already traced in the case of olivine-dolerite and described in this paper (see pages 372, 373). The dykes of this rock occur in association with those of olivine-basalt in the bed of the Sai river. It can, therefore, be definitely said that the two rock-types are the same and they differ only in the stage of alteration of augite. It is not clear as to why the whole of the augite in the olivine-basalt was altered, whereas that was not the case in the olivine-dolerite.

From their un-metamorphosed nature and correlation with similar rocks of the neighbouring Sirohee State, the olivine-dolerite and olivine-basalt of Danta State are assigned to Post-Erinpura-Granite age. No dykes or veins of these rocks have, however, been discovered traversing the Erinpura-granite of Danta State, though masses of Erinpura-granite occur in the neighbourhood of every outcrop of these rocks. It may be that the calc-series afforded easy channels for these intrusions which were comparatively very small in dimensions. The solid mass of Erinpura granite was probably too hard for these intrusions to cut through.

Conclusion.

The basic intrusives of Danta State can thus be classified into the following types according to their structural and mineralogical characters. These rock-types may also represent the three basic phases of igneous activity in this area during the Post-Aravalli period :—

1.	Oldest intrusives (Post-Aravalli, but Pre-Granitoid-Gneiss, in age).	Pyroxene-granulite, Epidiorite and Hornblende-schist.
2.	Older intrusives (Post-Granitoid-Gneiss, but Pre-Erinpura-Granite, in age).	Meta-gabbro and Meta-dolerite.
3.	Younger intrusives (Post-Erinpura-Granite in age).	Olivine-dolerite and Olivine-basalt.

The younger intrusives of olivine-dolerite and olivine-basalt afford an interesting study in the alteration and replacement of pyroxene.

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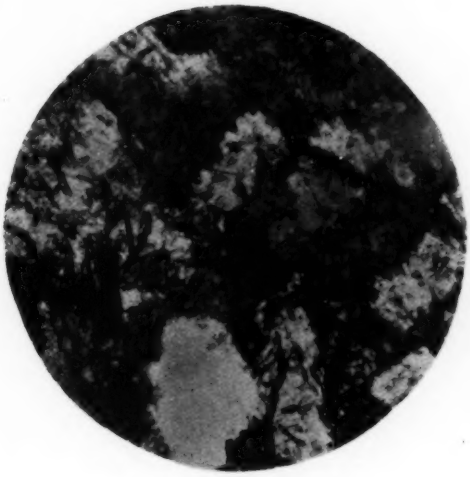
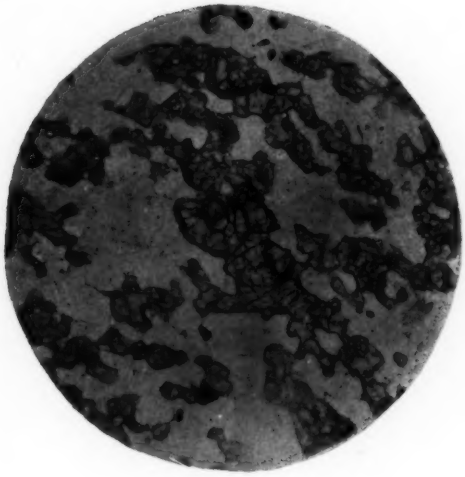


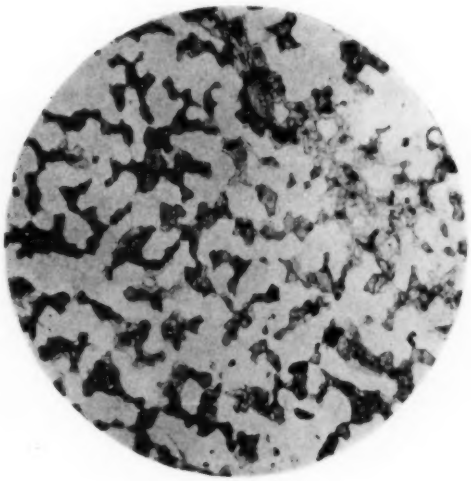
(Fig. 1.)
Epidiorite showing relict ophitic structure and absorption of alkali from felspar laths by hornblende which is more blue (here darker grey) along the margin. (Sp. No. D/9).
Plane polarised light × 20.

(Fig. 2.)
Hornblende-schist showing glomero-porphyroblasts of hornblende in a lepidoblastic groundmass (Sp. No. D/196).
Plane polarised light × 20.



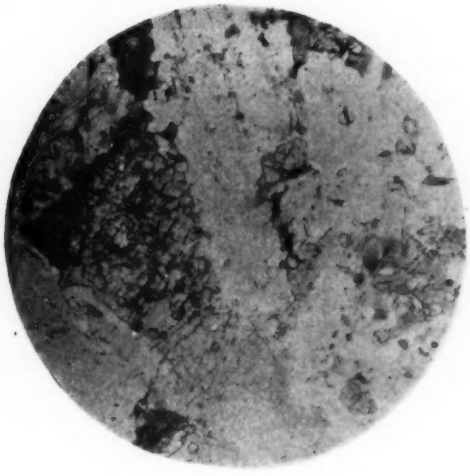
(Fig. 3.)

Pyroxene-granulite showing granulitic structure (Sp. No. D/379).
Plane polarised light × 20.



(Fig. 4.)

Garnet-granulite showing grossularite surrounded by epidote (Sp. No. $D^1/56$).
Plane polarised light × 20.



(Fig. 5.)
Meta-gabbro showing ophitic structure and pyroxene altering to hornblende (Sp. No. $D^1/51$).
Plane polarised light × 20.

(Fig. 6.)
Meta-gabbro showing ophitic structure, and cataclastic diallage (Sp. No. D/452).
Plane polarised light × 20.

(Fig. 7.)
Olivine-dolerite showing ophitic structure and titaniferous augite (Sp. No. D/545).
Plane polarised light × 20.

(Fig. 8.)
Olivine-dolerite showing interpenetration of laths of felspar (Sp. No. D[1]/61).
Nicols crossed × 20.

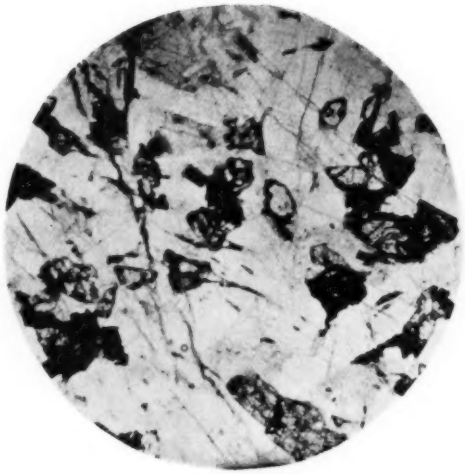
(Fig. 9.)

Olivine-dolerite showing ophitic felspar enclosing an aggregate of biotite, chlorite and magnetite (Sp. No. $D^1/63$).
Plane polarised light × 20.

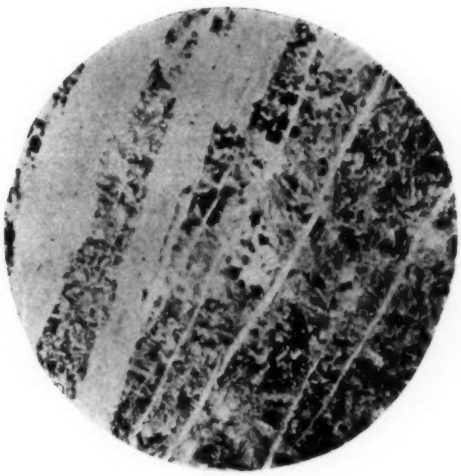
(Fig. 10.)

Olivine-dolerite showing amygdule-like calcite surrounded by chlorite (Sp. No. D/545).
Plane polarised light × 20.

(Fig. 11.)
Olivine-dolerite showing ophitic felspar enclosing triangular patches of pure calcite (Sp. No. $D^1/107$).
Nicols crossed × 20.

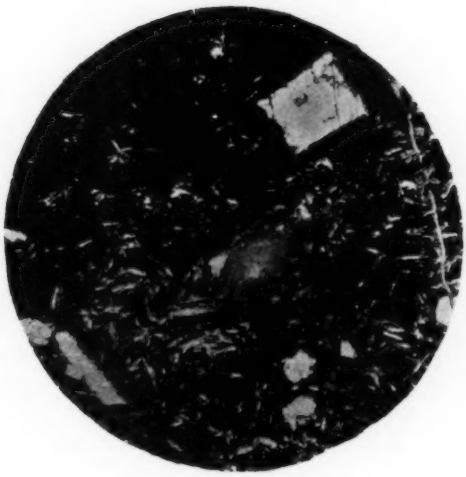


(Fig. 12.)
Olivine-dolerite showing crystals of altered olivine enclosed in felspar laths (Sp. No. $D^1/63$).
Plane polarised light × 20.



(Fig. 13.)
Olivine-basalt showing veinlets of calcite and needles and triangles of magnetite in the groundmass (Sp. No. D/105).
Plane polarised light × 20.

(Fig. 14)
Olivine-basalt showing laths of felspar, hexagonal section of serpentine and numerous amygdules of calcite (Sp. No. D/693).
Plane polarised light × 20.



(Fig. 15.)
Olivine-basalt showing microlites of felspar in the opaque groundmass, and rhomb of colourless olivine (Sp. No. D/446).
Plane polarised light × 20.

(Fig. 16.)
Olivine-basalt showing colourless olivine, ophitic felspar and almost opaque groundmass (Sp. No. D/446).
Plane polarised light × 20.

The Coloured Plate (No. III) refers to an article on *" Plasmodium narayani* N. Sp., Parasite of the Fish Otter *Lutra lutra"* by Col. I. Froilano de Mello and Emerciano Dias appearing in Vol. III, No. 3.

EMBRYOLOGICAL STUDIES IN THE LYTHRACEÆ.—III.

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This last part of these "Studies" deals with the structure and development of the ovule and the embryo-sac in *Ammania*, *Nesæa* and *Woodfordia*, and the development of pollen, male gametophyte, endosperm, embryo and seed in the family. At the end a summary is given of the chief embryological features of the Lythraceæ and a comparison is made with those of related families. The plants studied include, besides *Lawsonia inermis* and species of *Lagerstræmia* described in the earlier papers, four species of *Ammania* (*A. baccifera* Linn., *A. multiflora* Roxb., *A. pentandra* Roxb. and *A. peploides* Spreng.), *Nesæa myrtifolia* Desf. and *Woodfordia floribunda* Salisb. Out of the four species of *Ammania*, two (*A. baccifera* and *A. multiflora*) belong to the sub-genus *Eu-Ammania* and the other two (*A. pentandra* and *A. peploides*) belong to the sub-genus *Rotala*. The material of all the above species of *Ammania* was collected locally from wild plants and was fixed either in Allen's modified Bouin's fluid or in Navaschin's fluid. Material of *Nesæa myrtifolia* was taken from a plant cultivated in the Royal Botanical Gardens, Sibpur, Calcutta, and was fixed in Karpetschenko's fluid. Material of *Woodfordia floribunda* was partly obtained from a plant grown at the Royal Botanical Gardens and partly from wild plants growing in the Kumaon Hills, Himalayas, during the course of a botanical excursion in those hills. It was fixed in both cases in Allen's modified Bouin's fluid. Heidenhain's iron-alum-hæmatoxylin was mostly used for staining the sections.

I. *Structure and Development of the Ovule and the Embryo-Sac in* Ammania, Nesæa *and* Woodfordia.

This account is based on a study of all the plants mentioned above except *Ammania peploides*. It thus includes three species of *Ammania*, *Nesæa myrtifolia* and *Woodfordia floribunda*. Two species of *Ammania* (*A. baccifera* and *A. pentandra*) and *Nesæa myrtifolia* are fully illustrated in the paper. Of *Ammania multiflora* only a few important stages are

sketched, as it largely resembles *A. baccifera*. *Woodfordia floribunda* also is not fully illustrated here, as some observations on this plant have already been published by us elsewhere.[7] Here only those points are mentioned which were not at all or only inadequately described before.

Development of the inflorescence.—The development of the inflorescence has been studied in species of *Ammania* and fig. 1 clearly shows that the flower clusters in the axils of the leaves of *Ammania baccifera* develop in a cymose manner. The main axis is seen to end in the flower marked F_1 and a small flower F_2 is seen to arise from one side of it. In *A. pentandra* the flowers generally occur singly in the axil of the leaves, but sometimes a cymule of three also has been observed by us.

The gynæcium.—The placentation is marginal and axile in all the species except *Ammania baccifera*. In the latter species, the placentation is free central (figs. 2 and 3), but a column of tissue projects vertically inwards from the base of the style and closely approximates the apex of the free central placenta. To the naked eye or under a low magnification, the two may appear to be continuous. For this reason in the various taxonomic works and the Indian Floras the placentation is described to be axile, as it is in the family Lythraceæ in general, but microtome sections when examined under the microscope always show that the central placenta is quite free from the internal projection from the base of the style.

Although the placentation in *Ammania baccifera* is clearly free central, transverse sections commonly—we cannot say if always—reveal the presence of rudimentary septa of very short height just at the base of the ovary (fig. 5). There are 3 or 2 of these. When there are three, these are equally distributed. When there are only two, they are seen towards one side, as in fig. 5, clearly leaving the space for the third. This shows that the ovary in this species is tri-carpellary.

Fig. 4 illustrates an abnormal form of the free central placenta seen in *Ammania baccifera*. It was found to be much dichotomously divided, recalling to some extent the appearance of an abnormal case described by P. C. Joshi[10] in *Thylacospermum rupifragum*.

The style is bent upon itself in the bud stage in *Nesæa* just as in *Lawsonia*[8]. This condition is also seen in *Lagerstræmia*, though it was not mentioned in the earlier paper.

The ovule.—The ovules in every case are anatropous with the micropyle pointing downwards and possess two integuments. In *Nesæa myrtifolia*, however, the ovules near the base and the apex of the ovary are frequently amphitropous (figs. 51 and 52) and occasionally the micropyle of the ovules

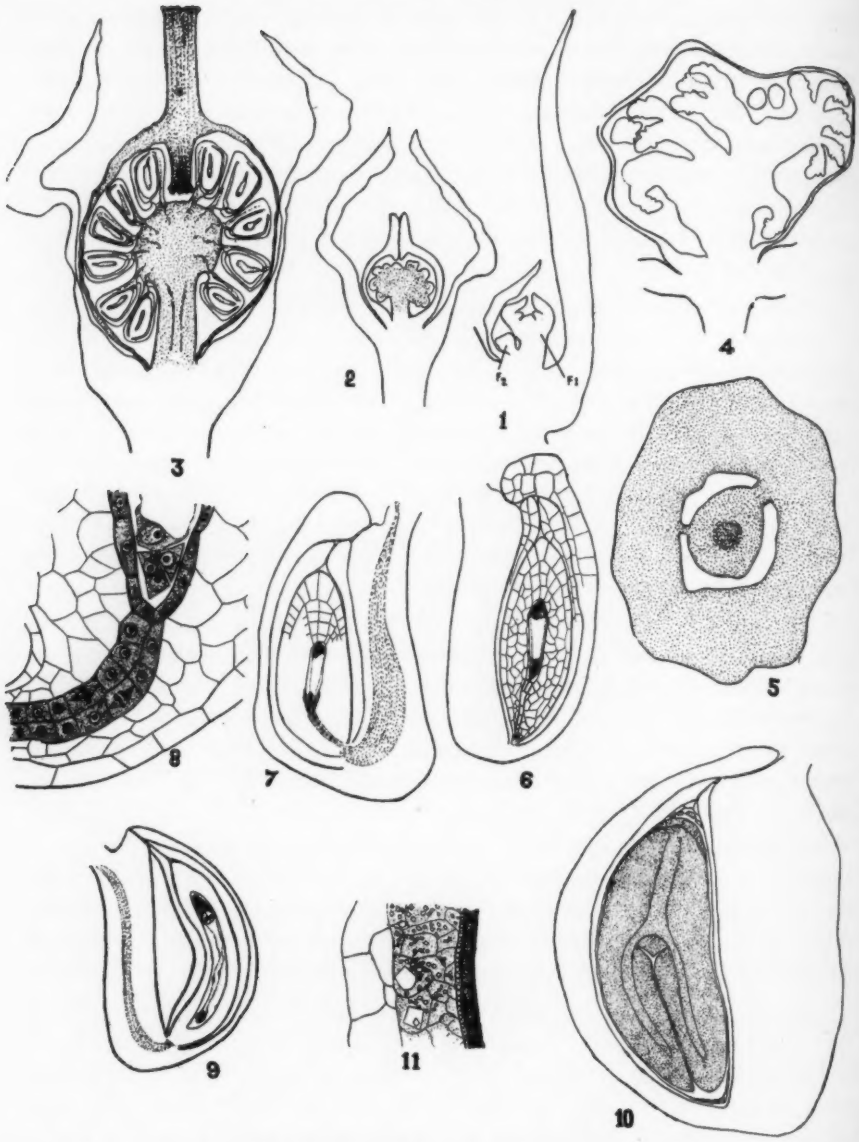
just at the base of the ovary points upwards (fig. 51). In one instance, an ovule was seen to be in an orthotropous condition at the megaspore-mother cell stage, when all other ovules of the ovary had assumed the anatropous form (fig. 50). This ovule also had, as an exceptional case, only a single integument. For a record of similar variations in other plants reference may be made to a paper by one of us published elsewhere (Joshi[5]). The ovules in all the forms show a slight flattening on the side of the raphe (fig. 7), especially near the apex, foreshadowing the large wing developed in seeds of *Lagerstræmia*.

Both the integuments take part in the formation of the micropyle, as in *Lawsonia*[8] and *Lagerstræmia*[9]. The portion of the micropyle formed by the outer integument is not in line with that formed by the inner integument (figs. 6 and 7). This is also seen in the ovules of *Lawsonia* and *Lagerstræmia*, though this point was not noted in the earlier papers. Thus the micropyle in all Lythraceæ appears to be somewhat zig-zag, as described by Samal[12] in *Crotalaria juncea*. During the flowering stage in all the three genera—*Ammania*, *Nesæa* and *Woodfordia*—the integuments are mostly two cells, or here and there 3 cells, thick and consist of uniform cells. Their structure in the seed is described afterwards.

The nucellus in the fertilisable ovules consists of about 5 layers of cells above the embryo-sac, about 3 or 4 layers of cells on the sides of the embryo-sac and of about 7–10 layers of cells below the embryo-sac. It shows a slight curve in the chalazal region as in *Lawsonia* and *Lagerstræmia* and a distinct strand of regularly arranged cells (figs. 7 and 8), connecting the vascular supply ending in the chalaza of the ovule with the base of the embryo-sac, just as in those genera.

Double nucelli.—Two nucelli within one ovule have been seen in *Nesæa myrtifolia*, just as in *Lagerstræmia indica*[9] and in species of *Cuphea*[11]. In one case (fig. 53) both the outer and the inner integuments were common. In the second example (fig. 54), the outer integument was common to both the nucelli, but each had a separate inner integument of its own.

Megasporogenesis.—Our observations on the primary archesporium in the ovules of *Ammania*, *Nesæa* and *Woodfordia* agree with those made on *Lawsonia*[8] and *Lagerstræmia*[9]. The primary archesporial cells are always many in number (figs. 12, 13, 14, 31, 55 and 66). While generally these cells are hypodermal, sub-hypodermal archesporial cells are quite common and have been found in every species investigated (figs. 12 and 66). A single archesporial cell has been exceptionally seen only in *Ammania pentandra* (fig. 32).

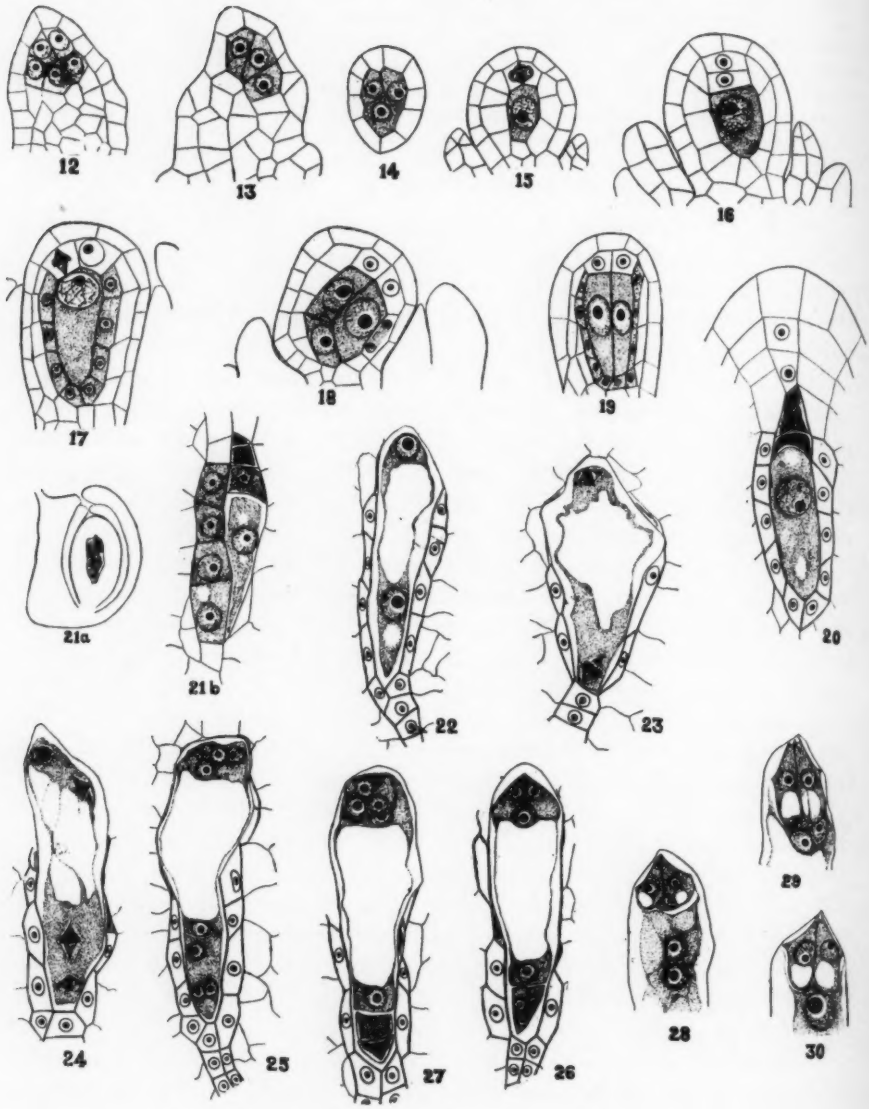


Figs. 1–11.—*Ammania baccifera*. Figs. 1-3, various stages in the development of the flower, especially gynæcium. Fig. 1 also shows the development of the inflorescence. The older flower (F_1) is terminal and the younger one (F_2) is seen arising from one side. Fig. 3 is a longitudinal section from an ovary containing nearly mature ovules and shows the free

central nature of the placentation. Fig. 4, longitudinal section of an ovary showing an abnormal much-divided free central placenta. Fig. 5, transverse section of an ovary near the base showing two septa. Figs. 6 and 7, longitudinal sections of ovules showing the form, micropyle, nucellus and the chalazal strand of conducting cells; fig. 7 along the raphe; fig. 6, a little towards one side of it. Fig. 8, the chalazal strand of conducting cells at a higher magnification. Figs. 9-10, two stages in the development of the seed; fig. 9 showing an embryo at the quadrant stage; fig. 10 showing a nearly mature embryo. Fig. 11, a portion of the testa from a nearly mature seed. Figs. 1, 2 and 4, × 260; Fig. 3, × 55; Fig. 5, × 90; Figs. 6 and 7, × 260; Figs. 9-10, × 125; Fig. 8, × 820; Fig. 11, × 315.

As in other plants of the family, one archesporial cell only cuts off a parietal cell and develops into the megaspore-mother cell and the other archesporial cells gradually lose their distinctive features and merge into the ordinary cells of the nucellus, but exceptions to this condition are very common and are seen in every species. Fig. 19 shows a case of two megaspore-mother cells in an ovule of *Ammania baccifera* lying side by side. These have developed from two adjacent archesporial cells after cutting off the parietal cells. Fig. 18 shows an ovule from the same plant with three sporogenous cells, two having taken origin from hypodermal archesporial cells and having cut off parietal cells, while the third has developed from a sub-hypodermal archesporial cell and has not cut off any parietal cell. Figs. 21*a* and *b* again illustrate from the same species an ovule in which two megasporocytes have even formed complete tetrads of megaspores. Figs. 34 and 36 illustrate two examples of double megaspore-mother cells from *Ammania pentandra*. In fig. 34, one of them has taken its origin from a hypodermal archesporial cell after cutting off a wall cell and the other from a sub-hypodermal archesporial cell and without cutting off a wall cell. The first is seen lying above the latter and both are in synizesis. In fig. 36, both the megaspore-mother cells are of hypodermal origin and have cut off parietal cells and one of them (on the left side in the figure) has already developed into a tetrad of megaspores. Fig. 44 shows an example of double megasporocytes developed from hypodermal archesporial cells after cutting off wall cells from *Ammania multiflora*. Fig. 58 shows the nucellus of an ovule from *Nesæa myrtifolia* with two megaspore-mother cells in synizesis, and lastly fig. 70 provides us with an example from *Woodfordia floribunda* with two megaspore-mother cells in nearly the same condition as described for *Ammania pentandra* in fig. 36.

The first division in the primary wall cell may be either anticlinal (figs. 15, 17, 34, 43 and 44) or periclinal (figs. 16, 36 and 57). This is followed by a number of divisions in all planes and ultimately results in the formation of 4 or 5 layers of parietal tissue above the micropylar end of the embryo-sac. A tapetum around the functioning megaspore-mother cell (or cells) is seen in species of *Ammania* (figs. 17, 19, 20), particularly in *Ammania baccifera*,



Figs. 12-30.—*Ammania baccifera*. Figs. 12-14, primary archesporium; in transverse section in fig. 14. Figs. 15-17, various stages in the development of the megaspore-mother cell and the parietal tissue. Fig. 18, three megaspore-mother cells in one ovule. Fig. 19, two megaspore-mother cells in one ovule. Fig. 20, a linear tetrad of megaspores. Fig. 21*a*, an

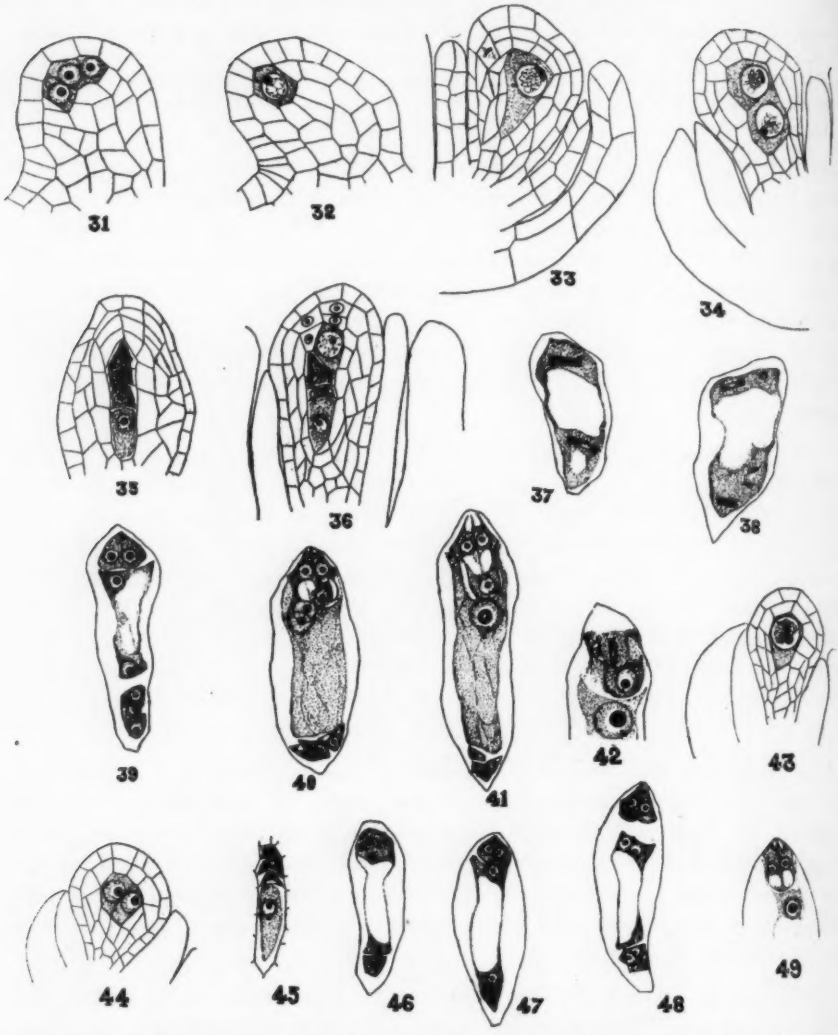
ovule with two tetrads of megaspores; fig. 21*b*, tetrads from the same at a higher magnification. Figs. 22-27, various stages in the development of the embryo-sac. Figs. 28-30, micropylar portions of the embryo-sac showing progressive stages in the differentiation of the egg-apparatus, particularly synergids and the secondary nucleus. All figs. except 21*a*, × 800; 21*a*, × 165.

but it is crushed during the development of the embryo-sac. The degeneration proceeds from the micropylar end towards the chalazal end, as the embryo-sac increases in diameter more in the first region. In other genera, such a layer of tapetal cells is only faintly differentiated from the other nucellar cells. Below the functioning megaspore-mother cell a row of axial cells is formed just as in *Lawsonia* and *Lagerstræmia*. These cells by their division ultimately give rise to the strand of regularly arranged cells connecting the chalazal end of the embryo-sac with the vascular bundle of the ovule.

The functioning megaspore-mother cell undergoes a long period of rest and increases considerably in size before it undergoes the two meiotic divisions. In every case it gives rise to a complete tetrad of megaspores which has generally a linear form (figs. 20, 21*a* and *b*, 35, 36, 45, 60, 68 and 70), but may be occasionally in *Nesæa* and *Woodfordia* T-shaped (figs. 61 and 69). Development of the tetrad has been seen by us in *Nesæa* and *Woodfordia* and it appears that the chalazal dyad cell generally divides before the micropylar dyad cell (figs. 59, 67 and 68). The megaspore which develops into the embryo-sac is always the chalazal one and the other three in these plants have always been seen to degenerate.

Development and structure of the embryo-sac.—Development of the embryo-sac from the functional megaspore takes place in the normal manner (figs. 22-26, 35-40 and 62-63), except that the bi-nucleate embryo-sac, as in *Lagerstræmia*, is often characterised by the persistence of the chalazal vacuole for an unusually long period (fig. 22) and sometimes this condition is seen even during the development of the 4-nucleate embryo-sac (fig. 37) or after its formation (fig. 62).

The antipodals are the first structures to differentiate in the embryo-sac (figs. 25 and 46). They generally show small or large vacuoles. In *Nesæa myrtifolia* and *Woodfordia floribunda*, as in other investigated Lythraceæ, they begin to degenerate very early and generally disappear by the time the polar nuclei fuse together (fig. 64). In species of *Ammania*, they have been found to be a little more persistent than in other members of the family and are often present in the embryo-sac up to the time of the formation of the secondary nucleus (fig. 41). Sometimes, however, even in this genus, they may degenerate very early (fig. 27). The polar nuclei which are at



Figs. 31-42.—*Ammania pentandra*. Figs. 31 and 32, primary archesporium. Fig. 33, megaspore-mother cell in synizesis. Fig. 34, two megaspore-mother cells in synizesis. Fig. 35, a tetrad of megaspores. Fig. 36, a tetrad of megaspores and a megaspore-mother cell lying in the same ovule. Figs. 37-41, various stages in the development of the embryo-sac. Fig. 42, micropylar portion of an embryo-sac at the time of fertilisation; a pollen-tube is seen with the generative nucleus in telophase. × 635.

Figs. 43-49.—*Ammania multiflora*; a few stages in the development of the embryo-sac. Fig. 43, megaspore-mother cell in synizesis. Fig. 44, two megaspore-mother cells. Fig. 45, a tetrad of megaspores. Figs. 46-48, three stages in the differentiation of the 8-nucleate embryo-sac. Fig. 49, mature synergids and the secondary nucleus. × 635.

first at either pole of the embryo-sac (figs. 26, 27, 39, 46 and 47) meet somewhere above the middle of the embryo-sac (fig. 28) and then move up to a position just below the egg-apparatus (figs. 29, 40, 48 and 63). Here they fuse and give rise to a large secondary nucleus before the pollen-tube enters the embryo-sac (figs. 30, 41, 49 and 64). During the development of the egg-apparatus, the synergids differentiate before the egg cell (figs. 26 and 27) and their nuclei are derived from a common mother nucleus. The nucleus of the egg and the upper polar nucleus form the second pair of sister nuclei, as in angiosperms in general. Our further observations on the structure of the egg-apparatus (figs. 28-30, 39-41, 47-49 and 63-65) agree with those made previously on *Lawsonia*[8] and *Lagerstræmia*[9]. The mature synergids have always got prominent hooks and, besides the large chalazal vacuole, show a small vacuole in their apex as well.*

The form and size of the mature embryo-sac in *Woodfordia floribunda* has already been described. In species of *Ammania*, it is about 50-60μ long and is comparatively broader at the micropylar end and narrower towards the chalazal end. In *Nesæa myrtifolia*, the embryo-sac at the time of fertilisation measures about 80-100μ and is nearly equally broad at both the ends (fig. 65).

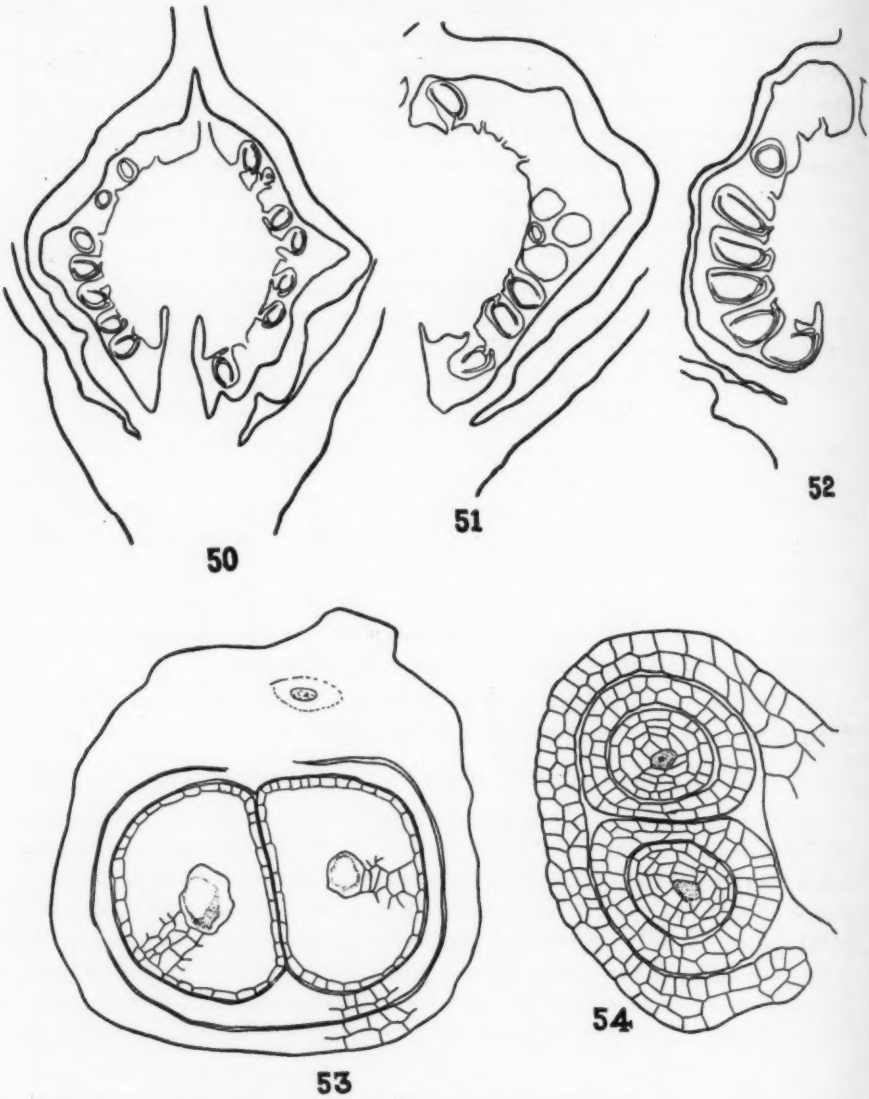
Degenerations in the embryo-sac, which are so commonly seen in *Lawsonia inermis*[8] and species of *Lagerstræmia*[9], are also observed in *Woodfordia floribunda*, but are absent in species of *Ammania*.

A case of reversed polarity in the embryo-sac was seen by us in *Woodfordia floribunda* and has already been described[7].

II. Development of Pollen and Male Gametophyte.

The development of pollen and male gametophyte has been followed in two species of *Ammania* (namely, *A. baccifera* and *A. pentandra*), *Lawsonia inermis* and *Nesæa myrtifolia* in all its stages, except the differentiation of primary archesporium, which has been observed only in preparations from *Ammania*. In the two species of this genus, the primary archesporium consists in each of the four lobes of the anther of a hypodermal row of 6 to 9 cells, as may be judged from longitudinal (fig. 72) and transverse (fig. 73) sections of the young anthers. The first division in the primary archesporial cells is

* Some previous observations published by us on the synergids of *Ammania baccifera* (6) are incorrect. These had resulted from a confusion of the synergids with the strong accumulation of endosperm (described later) formed in the micropylar part of the embryo-sac, which was caused by the absence of certain developmental stages in the material then examined and the presence of pollen-tubes in the embryo-sac even after the development of the endosperm had begun.



Figs. 50–54.—*Nesæa myrtifolia*. Figs. 50–52, longitudinal section (fig. 50) or portions of longitudinal sections (figs. 52–53) of the ovary showing placentation and the form of the ovules. Fig. 53, two nucelli within common outer and inner integuments. Fig. 54, two nucelli within a common outer, but separate inner integuments. Figs. 51–52, × 45; Fig. 53, × 375; Fig. 54, × 440.

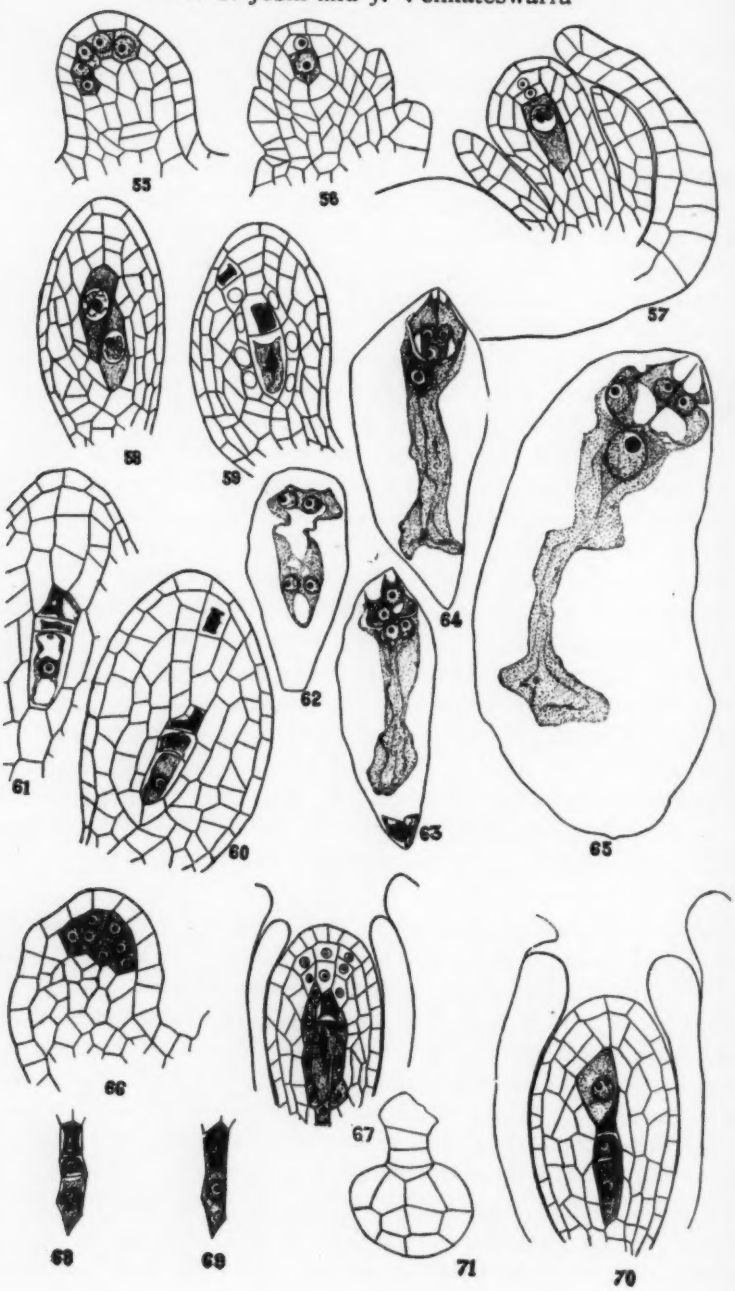
periclinal as usual (figs. 72 and 74) and leads to the formation of the layer of primary parietal cells towards the outside and primary sporogenous cells towards the inside. The first periclinal division in the primary parietal cells gives rise to two layers (fig. 74). The cells of the outer one of these do not generally divide any more periclinally, but those of the inner divide once more in a periclinal fashion and thus give rise to two layers of narrow flattened cells (figs. 75 and 76). In all the genera in this manner three layers of parietal cells are formed below the epidermis. The primary sporogenous cells undergo a number of divisions in all planes and give rise to a fairly large amount of sporogenous tissue.

As figs. 75 and 76 show, the tapetum seems to be derived largely from the outer sporogenous cells. Through its greater part it consists of a single layer of cells, but here and there it is even 2-seriate (fig. 76, at the points marked *t*). About the time that the microsporocytes enter synizesis, the nuclei of the tapetal cells undergo an ordinary mitotic division, just as Cooper[1] has recently demonstrated in so many other angiosperms, and become 2-nucleate. Most of the tapetal cells remain in this condition throughout their life, but the nuclei in some tapetal cells undergo further divisions, most probably of the amitotic type, and these tapetal cells become 3-6-nucleate.

The microspore-mother cells do not separate from one another and round off prior to the two meiotic divisions, but remain packed together (fig. 76). The meiotic divisions take place in the normal manner. At the end of the first meiotic division, an evanescent cell-plate is formed. At the end of the homotypic division, the grand-daughter nuclei become connected with one another by means of secondary spindle fibres. Cytokinesis takes place by furrowing. Pollen grains are formed in a simultaneous manner. They are generally arranged in a tetrahedral manner (fig. 81), but bilateral arrangement is also frequent.

During the formation of the pollen grains, the anthers undergo the usual increase in size and the epidermis of the anther-lobes and the layer of parietal cells just below the epidermis, which later on develops into the fibrous endothecium, keep pace with this growth by means of anticlinal divisions. The two layers of parietal cells between the fibrous endothecial layer and the tapetum are crushed early due to the growth of the latter. The tapetal cells begin to degenerate after the formation of tetrads and ultimately disappear. Here and there a tapetal cell may be seen lying in between the young pollen grains or tetrads, but no periplasmodium is formed.

The pollen grains, as usual after tetrad formation, separate and round off before the differentiation of exine and intine begins, but in one case in *Nesæa myrtifolia* they had failed to separate even when they had undergone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Figs. 55-65.—*Nesæa myrtifolia.* Figs. 55–56, primary archesporium and the cutting off of the primary wall cell. Fig. 57, megaspore-mother cell in synizesis. Fig. 58, a nucellus with two megaspore-mother cells. Fig. 59, nucellus showing development of the tetrad of megaspores. Fig. 60, a nucellus with a linear tetrad of megaspores. Fig. 61, a T-shaped tetrad of megaspores. Fig. 62, 4-nucleate embryo-sac; the chalazal vacuole is still present. Fig. 63, a young 8-nucleate embryo-sac. Figs. 64–65, older embryo-sacs, after the degeneration of the antipodals. × 600.

Figs. 66–71.—*Woodfordia floribunda.* Fig. 66, primary archesporium. Figs. 67–68, two stages in the development of the tetrad of megaspores. Fig. 69, a T-shaped tetrad of megaspores. Fig. 70, an ovule with one tetrad of megaspores and a megaspore-mother cell. Fig. 71, a stage in the development of the embryo. × 600.

their full development (fig. 81). The first division of the nucleus in the pollen grains (fig. 77) takes place after the exine and intine have become fully differentiated and the pollen grains have undergone most of their development in size, and is followed by the formation of a cell-plate and a lenticular generative cell is formed separated by a curved wall from the vegetative cell (figs. 78 and 81). Later on, the intervening wall between the two nuclei disappears and the pollen grains are in this 2-nucleate condition at the time of shedding (figs. 79 and 80). The division of the generative nucleus to form two male nuclei takes place just before fertilisation, at the apex of the pollen-tube, as it reaches the embryo-sac (fig. 42).

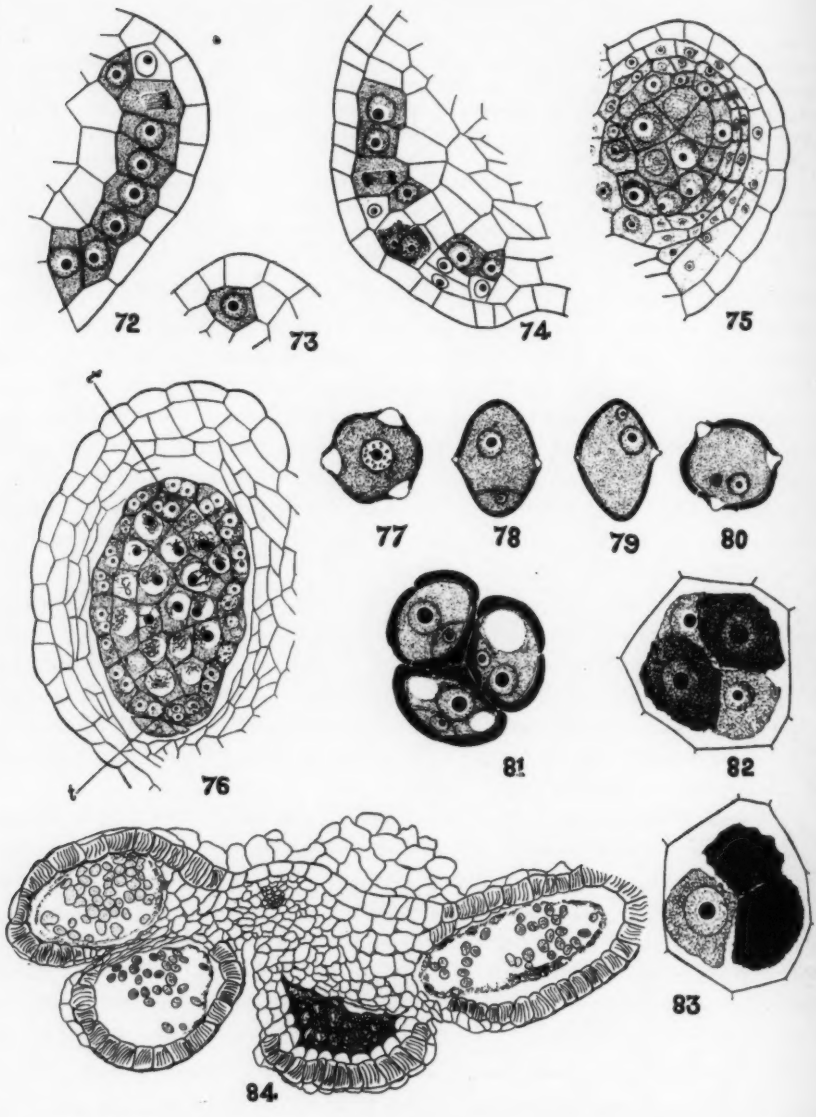
In form the mature pollen grains are nearly globose, but in *Ammania baccifera* they are slightly elongated, so that one diameter is slightly longer than the other at right angles to it (figs. 77-80). Sections of pollen grains along these different diameters, therefore, give different appearances and one may at first be wrongly led to believe that the pollen grains are dimorphic. There are generally 3 germ pores in the exine of each pollen grain arranged in an equitorial fashion (figs. 77-80).

Degenerations in the sporogenous tissue in the anthers are quite common both in *Nesæa* and *Lawsonia* and are also met with in species of *Ammania.* They occur during all stages of development,—in the primary archesporial cells (fig. 74), in the microspore-mother cells, in one, two, three (figs. 82 and 83) or all the pollen grains of a tetrad while inside the mother-cell wall, and in 1- and 2-nucleate nearly mature pollen grains. They may affect only a few cells of the loculus of an anther, but sometimes the whole loculus may be affected (fig. 84).

III. Fertilisation, Endosperm and Development of Embryo and Seed.

The above-mentioned phases of the life-history have been studied fully in the genus *Ammania,* particularly in two species *A. baccifera* and *A. pentandra,* and to some extent in *Woodfordia floribunda.*

Fertilisation.—The pollen-tube enters the ovule by way of the micropyle and fertilisation is porogamous. Some pollen-tubes have been seen in the



Figs. 72–73, *Ammania baccifera*, longitudinal and transverse sections respectively of two anther-lobes showing the primary archesporium. Fig. 74, *Ammania pentandra*, longitudinal section of an anther-lobe, showing two primary archesporial cells at the upper end, next a cell showing the first division of the primary archesporium, next a primary archesporial cell which has already divided into the primary wall cell and the primary sporogenous

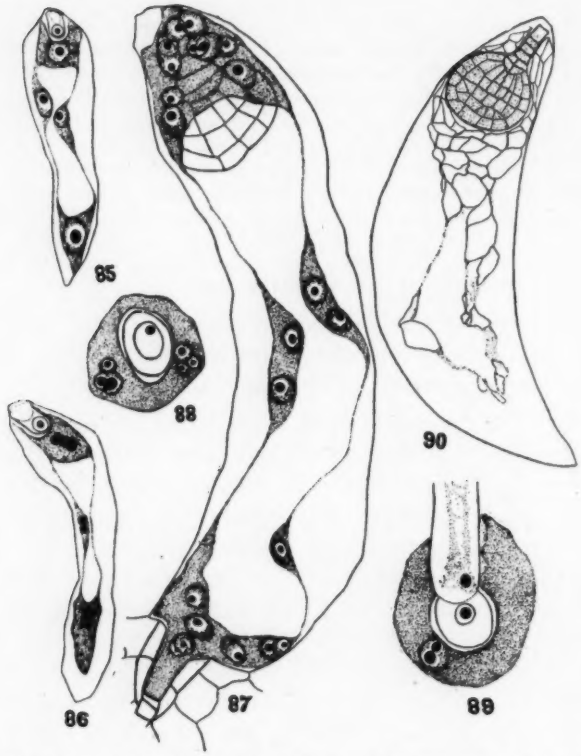
cell, next two degenerating primary archesporial cells, next the first periclinal division of the primary wall cell and lastly at the lower end a primary archesporial cell which has only completed the division into the primary wall and sporogenous cells. Fig. 75, *A. baccifera*, transverse section of an anther-lobe just after the differentiation of tapetum. Fig. 76, *A. baccifera*, transverse section of an anther-lobe with microspore-mother cells in synizesis. *t*, biseriate tapetum. Figs. 77-80, *A. baccifera*, pollen grains in various stages of development, in figs. 77 and 80 cut along the shorter diameter, in figs. 78 and 79 cut along the longer diameter. Fig. 81, *Nesæa myrtifolia*, an abnormal tetrad showing the full development of pollen grains without separating from one another. Figs. 82–83, *Lawsonia inermis*, pollen-mother cells showing degeneration of some young pollen grains while still inside the mother cell wall. Fig. 84, *Lawsonia inermis*, transverse section of an anther showing degeneration of one complete loculus. Figs. 72-73, × 940; Fig. 74, × 820; Figs. 75-76, × 480; Figs. 77-80, × 1440; Fig. 81, × 940; Figs. 82 and 83, × 1600; Fig. 84, × 145.

embryo-sac in *Ammania baccifera* even a long time after fertilisation and even when a good deal of endosperm has been formed (fig. 89). Double fertilisation has been observed in the same species.

Endosperm.—The formation of the endosperm begins before the first division of the oospore (figs. 85 and 86). Our observations on its development agree with those of Mauritzon[11]. The development takes place according to the nuclear type. After the first division of the primary endosperm nucleus, one of the daughter nuclei migrates to the chalazal end of the embryo-sac, while some daughter nuclei of the second reach the micropylar apex (fig. 85). An accumulation of cytoplasm now takes place at both the ends of the embryo-sac, while the protoplasmic lining on the sides of the embryo-sac remains very narrow. The nuclei that have reached the two ends of the embryo-sac divide very quickly by mitotic divisions (fig. 86) and soon accumulate in large numbers (figs. 87 and 88). In this manner chalazal and micropylar accumulations of endosperm develop. The micropylar accumulation of endosperm in *Ammania* is larger than the chalazal, just the reverse of what Mauritzon has described in *Peplis*. Fusions of nuclei in these accumulations of endosperm are commonly seen in later stages.

The endosperm remains free nuclear for a fairly long time, but ultimately becomes cellular as the three histogenic layers differentiate in the embryo (fig. 90). The large central vacuole, however, is never completely filled up with cells.

During the development of the endosperm, the embryo-sac undergoes great increase in length, and becomes slightly bent at both ends. At the chalazal end, in species of *Ammania*, a pouch-like structure is first formed, just as Mauritzon has figured in *Peplis portula*, piercing the chalazal strand of conducting cells (fig. 87), but after some time the end of the embryo-sac becomes even.



Figs. 85–90.—*Ammania baccifera*. Figs. 85–87 and 90, various stages in the development of the endosperm. Fig. 88, a transverse section of the micropylar accumulation of endosperm. Fig. 89, an oblique section of the micropylar accumulation of endosperm showing the presence of a pollen-tube even when a considerable amount of endosperm has been formed. Figs. 85–88, × 600; Fig. 89, × 770; Fig. 90, × 250.

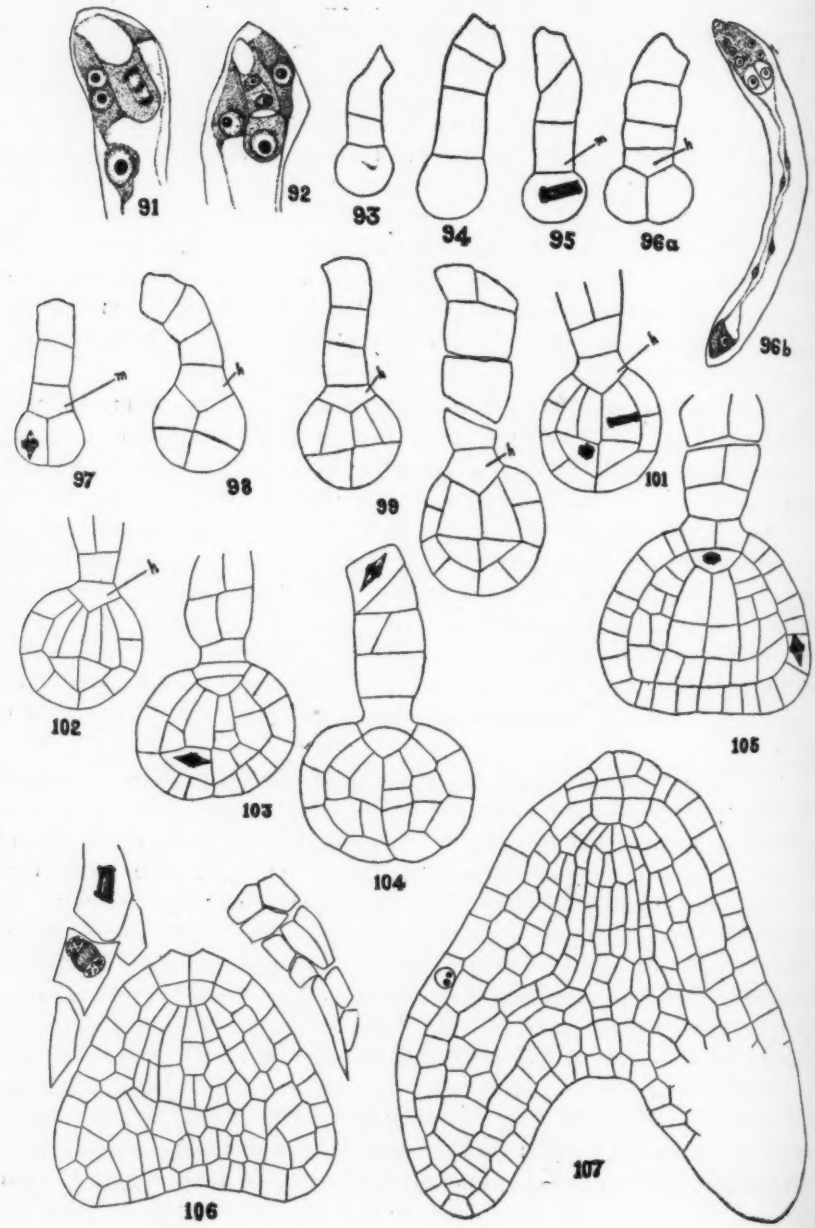
*Development of embryo**.—The development of the embryo agrees with that of *Lythrum Salicaria* as described by Souéges[14], except in small details, and corresponds to the "*Capsella*-type". The first division of the oospore is transverse (figs. 91 and 108) and this is followed by another transverse division in the basal cell (fig. 92). In this manner a row of three cells is formed (figs. 93 and 109). In *A. baccifera*, longitudinal divisions in the

* The development of the embryo in species of *Ammania* is very easy to follow. Sometimes a single cluster of flowers in *Ammania baccifera* may yield all the important stages. As the material of this genus is found throughout India, it can be recommended as a suitable type for study by various classes in preference to *Capsella*, the material of which is available only in certain parts of the country.

apical cell appear after this stage (fig. 110). In *A. pentandra*, one more transverse division takes place in the inferior-most cell before the appearance of the longitudinal walls in the apical cell (figs. 94 and 95). Thus the proembryo in *A. baccifera* consists of a row of three cells, while in *A. pentandra* generally of a row of four cells. In *Lythrum Salicaria*, the longitudinal divisions in the apical cell take place after the first transverse division of the oospore and the proembryo consists of only two cells. This is however not a point of a great difference between the two genera. As Souéges has shown, transverse divisions in the basal cell of the proembryo in *Lythrum Salicaria* take place afterwards. The difference consists only in the fact that these divisions in species of *Ammania* take place earlier. The same feature is seen in *Peplis portula*, *Ammania senegalensis* and *Nesæa syphilitica* studied by Mauritzon[11], and in these plants also the proembryo consists of 3 cells.

The terminal cell of the proembryo from an early stage assumes a more or less spherical shape and undergoes two longitudinal divisions in planes at right angles to each other and gives rise to the quadrants (figs. 96 and 111). This is followed by transverse divisions in each of the quadrants (fig. 97) and the octants are formed (figs. 98 and 112). The four superior octants give rise to the cotyledons and the stem-tip, while the four inferior octants give rise to the hypocotyl and the primary root except its apex. The hypophysis (marked *h* in figures) differentiates rather early during the development of the embryo, just as the quadrants (fig. 96) or the octants (fig. 112) are formed. It arises from the superior daughter cell of the penultimate cell of the proembryo (marked *m* in figures) and gives rise to the apex of the root.

The differentiation of the three histogenic layers proceeds just as in *Capsella* (figs. 99-107 and 113-116). It takes place earlier in the inferior octants. In the portion formed from the superior octants, the differentiation of plerome takes place very late,—after the differentiation of the cotyledons has taken place (fig. 107). The stem-tip differentiates very late, after the cotyledons have undergone a great part of their development, and remains even in the mature embryo a very small structure (fig. 117). The hypophysis cell divides first transversely giving rise to two cells, one superposed upon the other (figs. 103-104 and 114-115). Each of these divides by longitudinal walls to form a plate of four cells (figs. 106-107 and 118). The divisions in the cell next to the suspensor take place before those in the superior cell (figs. 105 and 116). The plate of four superior cells formed from the hypophysis completes the periblem at the apex of the root, while the four inferior cells complete the dermatogen and the root-cap (figs. 106–107 and 116 and 118).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Figs. 91–107.—*Ammania pentandra*. Longitudinal sections of various stages in the development of embryo. In figs. 91 and 92 the micropylar part of the embryo-sac is also shown. Fig. 96*b* shows the whole embryo-sac containing an embryo at the stage sketched in fig. 96*a*. In fig. 106 some of the endosperm cells surrounding the embryo are also shown. *h*, hypophysis; *m*, mother cell of the hypophysis. Figs. 91-96*a*, × 630; Fig. 96*b*, × 300; Figs. 97-107, × 630.

The suspensor is formed from the cells of the proembryo not taking part in the formation of the embryo proper and the inferior daughter cell of the penultimate cell of the proembryo. Some of these cells undergo a few transverse divisions, so that the suspensor becomes 4 or 5 cells long. In *Ammania baccifera*, it remains uni-seriate throughout its development, but in *A. pentandra* longitudinal walls also appear in all the suspensor cells, except in the sister cell of the hypophysis, so that through its greater length the suspensor becomes more than 1-seriate. The structure of the suspensor in *A. multiflora* agrees with that of *A. baccifera*, and that of *A. peploides* with that of *A. pentandra*. As has been mentioned before, the first two species belong to the sub-genus *Eu-Ammania* and the latter two to *Rotala*. The embryological findings thus support the current classification.

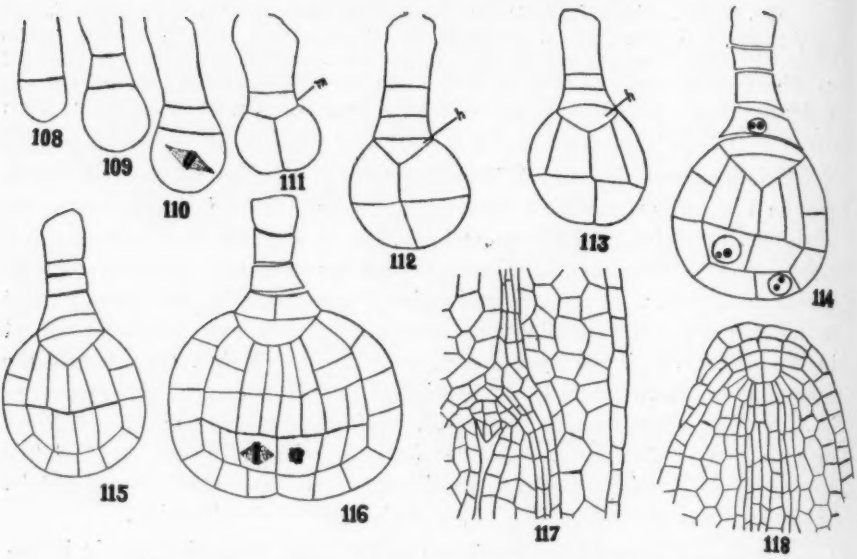
A few stages of embryo development have also been observed in *Woodfordia floribunda*, and as fig. 71 shows this agrees with the development in *Ammania* as described above.

Structure of the mature seed.—During its development, the embryo destroys the whole of the endosperm and later on the nucellus. The mature seed is thus exalbuminous (fig. 10). In the testa of the mature seed, the cells of inner layer of the inner integument become greatly thickened, while those of the outer layer do not undergo much change. Similarly, the cells of the outer layer of the outer integument also do not change very much, but those of the inner divide and give rise to about three layers of cells in which certain crystals are abundantly deposited (fig. 11).

During the development of the seed after fertilisation, the outer integument separates off from the inner on the side of the raphe. A large air-space thus appears in between the two (fig. 9). It has been seen in all species of *Ammania* mentioned above. The purpose of this space is probably to help in aeration during a period of great metabolic activity, as all these species grow in marshy places. During further growth of the seed, this air-space gradually disappears (fig. 10).

IV. Summary of the Embryological Features of the Lythraceæ.

The ovule.—The ovules in the Lythraceæ are generally anatropous and ascending. Only exceptionally they are found to be descending. In *Nesæa* only some of the ovules have been seen by us to have an amphitropous form.



Figs. 108-118.—*Ammania baccifera*. Figs. 108-116, longitudinal sections of various stages in the development of the embryo from the two-celled stage onwards. Fig. 117, a portion of the mature embryo showing the stem-tip and portions of cotyledons. Fig. 118, a portion of the mature embryo showing the root-apex. *h*, hypophysis; *m*, mother cell of hypophysis. × 780.

There are two integuments and both take part in the formation of the micropyle, which has been found to have in every case a somewhat zig-zag and not straight form.

The chief feature of the nucellus is the presence of a strand of regularly arranged, somewhat thick-walled cells in the chalazal region, connecting the vascular bundle of the ovule ending in the chalaza with the base of the embryo-sac. We have not performed any chemical tests to see if these cells are lignified, but there is little doubt that this strand corresponds to the hypostase described in the Onagraceæ[3] and other Myrtifloreæ. The probable function of this strand of cells is to conduct food materials to the embryo-sac.

The occurrence of two nucelli within one ovule seems to be quite common within the family. Mauritzon[11] has noted this condition in *Cuphea lanceolata* and *Cuphea petiolata*. We have noted this in *Lagerstræmia indica*[9] and *Nesæa myrtifolia*. These double nucelli may have common outer and inner integuments, or the outer integument alone may be common, and each nucellus may have a separate inner integument.

The female archesporium.—The primary archesporium in the ovule in all the forms investigated by us has been found to be multicellular. A single archesporial cell has been seen only occasionally in the genus *Ammania*. Mauritzon[11], however, has described a single-celled primary archesporium as characteristic of the family. These observations to our mind appear to be based on inadequate study of the early stages.

That the multicellular primary archesporium is the general condition in the family is supported by the common occurrence of two or three megaspore-mother cells or tetrads of megaspores and in some cases, as in *Lawsonia* and *Lagerstræmia*, even of more than one embryo-sacs in the same ovule. There is not a single species investigated by us in which we have not come across, and also in a fair number of cases, two or three archesporial cells cutting off the parietal cells and developing into megaspore-mother cells.

Among the allied families, according to Schnarf[13], a multicellular primary archesporium has been described in the Rhizophoraceæ (*Rhizophora Mangle, R. mucronata, Ceriops Candolleana* and *Bruguiera* species) by Karsten, Nyssaceæ (*Davidia involucrata*) by Horne, and a number of Onagraceæ (species of *Epilobium, Godetia, Oenothera*, etc.) by Michaelis, Schwemmle, Täckholm, Håkansson, etc. Recently in *Callistemon linearis* (Myrtaceæ), Tiwary and Rao[16] have also noted the occasional occurrence of multicellular primary archesporium. Some authors, however, doubt the presence of such multicellular archesporium. For instance, Johansen[4] writes, " I am constrained to regard with scepticism some of the published accounts of ' several archesporial cells ' in various onagrads." From what, however, we have found in the Lythraceæ, we think that these accounts are most probably correct and the occurrence of double or triple embryo-sacs in some Onagraceæ (Ishikawa[3]) supports such a conclusion.

Megasporogenesis.—The functional archesporial cells always cut off a parietal cell except when they are sub-hypodermal in position. The amount of parietal tissue formed varies in the different genera.

A linear tetrad of megaspores is commonly formed, but occasionally T-shaped tetrads also occur, as has been seen in *Lawsonia, Nesæa* and *Woodfordia*. During the development of the tetrad, often the micropylar dyad cell divides later than the chalazal. According to Mauritzon[11] in some forms (*Peplis portula, etc.*) the nuclear division in the upper dyad is not followed by the formation of a cell-wall, so that a 2-nucleate micropylar megaspore is formed, or sometimes even the nuclear division in the upper dyad is suppressed. We have, however, not come across any such case in the forms investigated by us.

The chalazal megaspore of the tetrad is always the functional one. Only exceptionally in *Lawsonia inermis*[8] we have seen the second megaspore from the chalazal end enlarging and sometimes developing into the embryo-sac. In no case the micropylar megaspore has been seen to develop into the embryo-sac, although this is the fixed condition in the Onagraceæ.

Embryo-sac.—The chief noteworthy feature in the development of the embryo-sac is the persistence of the chalazal vacuole during the 2- and 4-free-nucleate stages. This has been seen in many forms both by Mauritzon[11] and by us. Characteristic of the mature embryo-sac is the early degeneration of the antipodals. This has been seen in every species investigated by us and other recent authors. Both these points, besides a few other minor ones, have been regarded by Tischler[15] and Mauritzon[11] to indicate that the embryo-sac of the Lythraceæ forms phylogenetically an intermediate stage between the 4-nucleate embryo-sac of the Onagraceæ and the normal 8-nucleate embryo-sac. To these arguments we may now add the various stages in the reduction of the antipodal nuclei described by us in *Lawsonia inermis*[8] as forming a still stronger connecting link.

The structure of the synergids in the Lythraceæ is characteristic in that they always develop a prominent hook and in later stages a small vacuole in their apex besides the large chalazal vacuole. The apical vacuole takes the place of the 'filiform apparatus' seen in other plants and serves probably the same function, namely, that of secreting chemotropically active substances which guide the pollen-tube.

During the secondary elongation after fertilisation, or sometimes it may be even before fertilisation, of the embryo-sac, the antipodal end often grows either into a pouch-like structure penetrating the chalazal strand of conducting cells (*Ammania* and *Peplis*) or forms a postament around the chalazal strand due to the resistance offered by the latter (*Cuphea* and *Lagerstræmia*)*.

Pollen and male gametophyte.—The primary archesporium in each lobe of the anther differentiates as a single row of hypodermal cells. Between the tapetum and the epidermis, typically three layers of cells are formed. The outer one of these forms the fibrous endothecium. The other two disorganise. Tapetal cells are 2-6-nucleate. They may have (*Lawsonia*, *Cuphea*, etc.) or may not have (*Ammania*, etc.) prominent vacuoles. Periplasmodium formation does not take place. A generative cell in the pollen grains is always formed at first, but later on the wall separating it from the

* The latter feature is also seen in the Myrtaceæ (*Callistemon linearis*), as is evident from the figure of Tiwary and Rao.[16]

vegetative cell dissolves. The pollen grains are 2-nucleate at the time of shedding and possess three germ spores arranged in an equatorial plane. The division of the generative nucleus to form two male nuclei takes place rather late, just before fertilisation.

Fertilisation.—Fertilisation is porogamous. Double fertilisation has been seen in *Ammania.*

Endosperm.—The endosperm in the Lythraceæ develops in the free nuclear manner. It has been found to be scanty in all the investigated forms and in this respect the family agrees with the Onagraceæ. Micropylar and chalazal accumulations of endosperm are commonly formed during the early stages.

Embryogeny.—The development of the embryo takes place according to the "*Capsella*-type". The chief difference consists in the early differentiation of the hypophysis. The development differs from the Onagraceæ in the origin of the hypophysis. In Onagraceæ, the hypophysis is formed directly from the penultimate cell of the proembryo. In Lythraceæ, it is formed from the superior daughter cell of the penultimate cell of the proembryo.

Within the family Lythraceæ, the different forms vary in the form of the suspensor. The suspensor remains throughout uni-seriate in *Peplis, Nesæa* and some species of *Ammania* belonging to the sub-genus *Eu-Ammania.* It has been found to become multi-seriate in the genus *Cuphea* and some species of *Ammania* belonging to the sub-genus *Rotala.*

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MARKED PERIODICITY IN REPRODUCTION OF THE PANJAB FRESHWATER ALGÆ.

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I made a regular collection of the Panjab Freshwater Algæ from July 1929 to May 1930, and since then I have been collecting algæ from December 1934 to December 1935 in Saharanpur District, which is on the boundary line of Eastern Panjab and Western part of the United Provinces and has a climate not very different from the eastern submontane districts of the Panjab, like Hoshiarpur and Gurdaspur. My two years' experience has shown me that there is a marked periodicity in the reproduction of spore-forming Chlorophyceæ which is closely connected with temperature and rainfall conditions.

A passing reference may also be made about those Chlorophyceæ which do not form spores with hard walls. Of these *Hydrodictyon reticulatum* is the commonest in ponds and slow-flowing freshwater streams. Its glistening daughter colonies may be found in nearly all months of the year, when water is found in the ponds and streams, and I have collected it in all stages of development in all months from July to February. I am excluding from consideration here all algæ which are found attached to artificial water-reservoirs and only those are discussed which are found in natural ponds and streams.

Seasons of Northern India.—I have divided a year in Northern India into five seasons: Hot Summer Months, Rainy Season, Autumn, Winter and Spring. Start may be made with the Hot Summer Months which begin from about 15th May and terminate by the middle of July. These months are characterised by dry heat. June is the hottest month of the whole year with a mean temperature of 89°.7 F., while the maximum may be as high as 107°.0 F. In these hot dry months, nearly all the ponds dry up, and only a few streams show a slow trickle of water. Due to absence of water no algæ can be seen in any ponds, while in some perennial streams *Œdogonium* may be found in a vegetative condition. Most of the algæ tide over these hot and dry months in the form of thick-walled spores.

II. *Rainy Season*—This may roughly be taken as beginning from the middle of July and ending by the second week of September. Due to the Monsoons, the temperature falls to 85° F. About 7 inches of rain falls in these two weeks, and the ponds and streams become fairly full with water. The maximum amount of rain falls in August, *i.e.*, about 8.5 inches, and all the ponds and streams overflow with water. The water of most of the ponds is usually turbid and muddy in August. The rainy season finishes by the second week of September, the rainfall in these two weeks seldom exceeding two inches, and mostly falling in showers. This is the halcyon season for the Myxophyceæ. On logs of wood light bluish patches of *Aphanocapsa montana* may be commonly seen. *Campylonema Lahorense* is found mixed with grass in dark brown patches almost everywhere. In water-troughs, bluish-green fascicles of *Schizothrix Mexicana* may be commonly seen intermixed with *Cladophora* and *Rhizoclonium*. On moist pieces of wood, green covering of Pleurococcus may also be seen. In some streams, young filaments of *Schizothrix* and *Compsopogen* may be seen, the latter being of purple colour, attached to reeds submerged in flowing water. Young filaments of *Spirogyra* are also seen and in most cases the chloroplasts do not show full maturity. The same may be said about various species of *Zygnema* and *Œdogonium*. It is with considerable difficulty that the last mentioned algæ may be found even in vegetative condition.

III. *Autumn Months*.—These may be taken as beginning with the third week of September and finishing by the middle of November. In the latter part of September the sides of ponds begin to dry up. On the sides of drying ponds *Botrydium* and *Protosiphon* appear in a thick green carpet. In the month of November, *Protosiphon* grows in large numbers on fields which have been lying fallow, in bright green patches, 2–8 yards in diameter. Cyst-formation in the subterranean rhizoidal portion takes place in a week, and then the algæ disappears from view. *Spirogyra* is also seen in a fertile condition in most ponds. I recorded four species of *Spirogyra* in this month, *viz.*, *Spyrogyra condensata*, *S. rivularis*, *S. crassa* and *S. nitida*.

IV. *Winter Months*—By the middle of November it becomes fairly cold in Northern India, and the mean temperature drops to 50°.0. The ponds are usually half full at this time, and the streams and rivers have a regular flow of clear, sparkling, and ice-cold water of the Himalayan snows. In some cold freshwater streams like the Siah Baeen near Jullunder with a swift current of water, *Chantransia chalybea*, *Batrachospermum moniliforme*, *Stigeoclonium variable* and *Cosmopogon* sp. are found attached

to blades of *Typha* in the mid-current. These members of Rhodophyceæ and *Stigeoclonium variable* are provided with well-developed basal cells for attachment. These algæ disappear in December, perhaps due to excessive cold, for the mean temperature may be as low as 45° F. In slowly running streams *Cladophora glomerata* may be seen in big tassels, looking like fox-tails attached to water-plants. All these algæ require a good deal of aeration, and I have noticed that where the current of water becomes very slow, these algæ tend to disappear. Here we may mention the peculiar case of *Cladophora glomerata* which may be seen in the tanks of the Shalimar Gardens, Lahore, growing on shells of Gastropods, which move about in the tanks, and thus aerate the algæ. However, in this case the growth of the alga is not luxuriant, and it appears to be a mere shadow of the *Cladophora glomerata* which grows in running streams. Due to slow locomotion of the Gastropod, the alga becomes thickly encrusted with Diatoms and dust particles. *Cladophora glomerata* also occurs in the water-reservoirs of wells fitted with Persian wheels, where it is constantly aerated by the flow of water from the well.

In December we usually have a rainfall of 1.5 to 2 inches especially in the last two weeks. After the dry months of October and November, this is very welcome, and gives a new lease of life to the algæ growing in ponds. In January the rainfall is more copious and is seldom less than 4.0 inches, and as the temperature is low and evaporation is less, the ponds become fairly full. The months of January and February are ideal for the growth of algæ. Along with the species of *Spirogyra* already mentioned I also found nine other species of the genus *Zygnema* which henceforth is usually seen only in a vegetative condition, can be found in a fertile condition. The commonest species of *Zygnema* is *Z. chalybeosporum* which is seen with plenty of zygospores. *Mougeotia genuflexa* may also be found at this time. Species of *Œdogonium* which I never noticed with ripe oospores in any of the above-mentioned months, produce sex organs with great regularity in the month of February, and most of the filaments show green oogonia and antheridia in the case of macrandrous forms, while the nannandrous forms show numerous androsporangia and nannandria growing on the walls of oogonia.

With the coming of winter-rains in December *Vaucheria sessilis* and *V. geminata* may also be seen in the form of bright green felt-like mats in lawns, gardens and other moist places. Oogonia and antheridia begin to appear in the first week of January, and by the last week of February nearly all the filaments become fertile, being heavily laden with oogonia, antheridia and oospores. *Vaucheria sessilis* is found both in aquatic and

terrestrial habitat, and it is curious that specimens of it collected from ponds at about the same time as from the lawns, proved to be more fertile, each filament being loaded with huge crops of oogonia and antheridia, while in the case of terrestrial specimens very few sex organs were seen.

V. *Spring Months*—Spring season may be taken as beginning with the first week of March. As compared with February there is a marked rise in temperature, the mean temperature in March being 62°.0 F. as compared with 49°.7 F. in February. Just as in spring a young man's fancy is supposed to lightly turn to thoughts of love, most of the algæ also show great reproductive activity. Various species of *Œdogonium* produce heavy crops of oogonia and antheridia, and by the end of March their filaments are laden with red, yellow, and chocolate-coloured oospores. By the middle of April nearly all species of *Œdogonium* bear ripe oospores. I recorded fourteen species of *Œdogonium* in these months all in a fertile condition. *Sphæroplea annulina* also shows numerous green ova in its oogonia in the beginning of March. By the end of March the ova become fertilised and young oospores, green in colour, enclosed in a thin membrane, may be commonly seen. In April the oospores become brick-red in colour with a thick hyaline wall produced into 10–15 spines, and arrayed in three alternate rows in the oogonia. This alga shows such an abundance of red oospores in April that many ponds appear deep red in colour. *Ghosella indica*, a new member of Conjugatæ, described by me as combining the characters of *Debarya* and *Zygnema*, with certain peculiarities of its own, is also found free-floating in dark green masses in the month of March. By the middle of April, *Ghosella* is all fertile and its conjugation canals bulge out with ripe zygospores of an orange-yellow colour. *Sirogonium sticticum* also produces zygospores in April.

Periodicity in Reproduction.—By the end of May most of the ponds begin to dry up, as there is no rainfall ; and the mean temperature rises to 81°.0 F. Now the algæ have to face the hot dry spell of three months beginning with May and ending with the middle of July. To meet this contingency, species of *Œdogonium*, *Spirogyra*, *Vaucheria*, *Zygnema*, *Ghosella*, *Sirogonium*, and *Sphæroplea* have produced thick-walled oospores which are capable of surviving the high temperatures of June and July. When the rains start in the middle of July, these oospores begin to germinate and produce young filaments. Then for the next four to five months, most of these algæ show a great vegetative growth with very little reproductive activity. The month of March provides optimum conditions for their reproduction, and probably this may be due to rise of temperature in March after the cool months of January and February. Thus there is a marked

periodicity in reproduction shown by these algæ in Northern India which is connected with more or less distinctly marked seasons. This is a remarkable phenomenon which is not noticeable in England or other temperate countries where the seasons are not so well marked, as we have in Northern India.

Season	Months	Minimum Temperature F.	Maximum Temperature F.	Mean Temperature 8·00 A.M.	Rainfall	Condition of ponds and streams	Algæ collected	Remarks
I. Hot Summer Months	15th May	73·4	105·4	86·4	..	Nearly all ponds and tanks dry up.	*Œdogonium* sp. *Cladophora glomerata.*	Œdogonium found in perennial streams only.
	June	80·1	107·4	89·7	..			
	15th July	80·2	100·5	87·1	..		*Rhizoclonium.*	
II. Rainy Season	15th July	80·2	100·5	87·1	7·5″	Ponds and tanks half full. Water turbid.	*Hydrodictyon reticulatum.* *Cosmopogon.*	
	August	78·9	97·8	85·2	8·5″	..	*Aphanocapsa montana* *Volvox aureus.*	Very few algae with thick-walled oospores are seen in these months.
	15th September	73·4	98·3	81·8	1·5″	All ponds full. Water clears up; the rudiment settles down.	*Campylonema Lahorense.* *Schizothrix mexicana.* *Oscillatoria princeps.* *Cylinderospermum stagnale.* *Schizomeris irregularis.* *Ulothrix oscillarina.*	
III. Autumn	15th September	73·4	98·3	81·8	..	Water present in most of the deep ponds.	*Botrydium granulatum.* *Protosiphon botryoides.*	Spirogyra becomes fertile.
	October	60·3	94·9	70·8	..	Shallow ponds dry up.	*Pandorina morum.* *Pithophora Kewensis.* *Stigeoclonium lubricum.* *Ulothrix.* *Cladophora Glœotrichia.* *Charaf Nitella.* *Aulosira fertilissima.* *Aphanocapsa serpentina.* *Spirogyra nitida and S.condensata.* *S. rivularis.* *S. crassa.* *Rivularia natans.*	
	15th November	48·2	83·9	57·0	..			

Season	Months	Minimum Temperature F.	Maximum Temperature F.	Mean Temperature 8·00 A.M.	Rainfall	Condition of ponds and streams	Algæ collected	Remarks
IV. Winter	16th November	48·2	83·9	57·0	..	..	*Zygnema* sp. *Batrachospermum moniliforme* *Chantransia chalybea.* *Stigeoclonium variable.* *Aphanochæte bulbochæte.* *Pithophora cactoides.* *Vaucheria uncinata.* *Draparnaldia glomerata.* *Zygnema chalybeosporum.* *Vaucheria sessiles.* *V. germinata.* *Nodularia spumigena.* *Spirogyra neglecta.*	*Rhodophyceæ* show a luxuriant growth in cold freshwater streams.
	December	40·9	72·8	45·3	1·5″	..		
	January	41·0	68·4	45·0	4·0″	Ponds fairly full.		
	February	44·5	72·1	49·7	..	..		
V. Spring	March	54·5	83·9	62·0	..	Ponds about half full.	*Sphæroplea annulina* *Sirogonium sticticum.* *Ghosella indica.* *Oedogonium urbicum.* *Oe. cardiacum.* *Oe. inerme.* *Oe. sociale.* *Oe. hirnii.* *Oe. gracillimum, Oe. oblongellum.* *Oe. pisanum.* *Zygnema chalybeosporum.* *Coleochæte Sontata* *C. Soluta.* *Nodularia spurmigena.*	Nearly all algæ with thick-walled oospores and zygospores are in a ripe condition in these months.
	April	64·5	96·5	75·4	2·0″	..		
	15th May	73·4	105·4	86·4	..	Water at the bottom of deep ponds and tanks only.		

GENUS "*ANABAENOTHRIX*" AND PARALLELISM IN EVOLUTION IN FRESHWATER ALGÆ.

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(Communicated by Dr. H. Chaudhuri.)

During my investigations of the Panjab Freshwater Algæ, I came across two *Anabæna*-like species of Blue Green Algæ, which, however, differed from the typical specimens of *Anabæna* in having a number of trichomes enclosed in a single mucilaginous sheath. Recently Dr. F. E. Fritsch described a species of *Anabæna* with a mucus sheath, from South Africa which he calls *Anabæna vaginicola* sp. nov.* This so-called *Anabæna vaginicola* Fritsch resembles *Anabæna cylindrica* Lemmer in essential features and the only vital difference is the occurrence of a number of trichomes within a single sheath. The two species which I discovered differ from *Anabæna vaginicola* Fritsch in the size of the filaments, the shape and size of the vegetative cells, and the form and size of spores and heterocysts. Following is a detailed description of these three species of *Anabæna*, like Blue-Green Algæ.

I. *Anabænothrix vaginicola* Fritsch (nov. comb.).—Numerous trichomes in a single sheath, rarely a single one in single sheath. Cells elongate cylindrical or sub-cylindrical 4 to 5 μ broad, 6 to 10 μ long. Spores oblong, barrel-shaped, contiguous to the heterocysts in series 4–5, 6.5 to 10 μ broad and 12 to 17.5 μ long.

II. *Anabænothrix cylindrica* Randhawa and Ghose.—Numerous trichomes enclosed in a single sheath, breadth of filaments 60–75 μ inclusive of the sheath, cells rounded or constricted, dumb-bell-shaped, 5 μ broad, 6–7 μ long with homogeneous contents. Heterocysts rounded 9 to 10 μ in diameter. Spores in pairs contiguous to the heterocysts, cylindrical in shape 4 to 5 μ broad, 18–20 μ long. Found attached in mud of a pond in long cylindrical columns of blue-green colour, which later on become detached, and the alga becomes free floating. Fig. 2 shows a filament of *Anabænothrix cylindrica* with numerous trichomes enclosed in a single

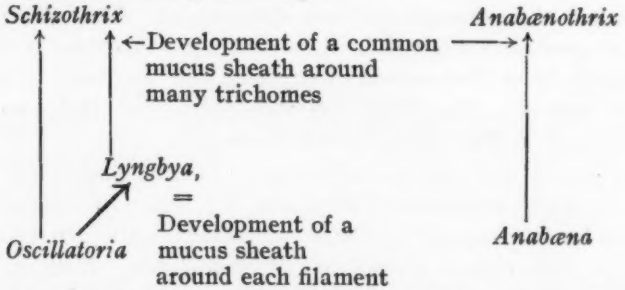
* *Trans. Roy. Soc. of S. Africa*, **18**, Parts 1 and 2.

sheath. In Fig. 2 is shown a part of a trichome highly enlarged with a heterocyst surrounded by a chain of spores on both sides.

III. *Anabænothrix epiphytica* Randhawa and Ghose.—Numerous trichomes enclosed in a single sheath or a single one in a single sheath. Cells rounded with homogeneous contents. 3.5 μ in diam. Heterocysts ellipsoid-rounded, always away from the spores. 5–6 μ broad, 14–18 μ long. Always found epiphytic on other algæ especially *Sirogonium*. In Fig. 3 is shown a typical filament of *Anabænothrix epiphytica* with a number of trichomes enclosed in a single sheath. Sometimes we come across filaments of this species, showing a single trichome enclosed in a sheath as in Fig. 4. In Fig. 5 is shown a part of a trichome with a spore and a heterocyst.

So here are three species of *Anabæna*-like Blue-Green Algæ which have acquired in the course of their evolutionary progress, the same character of enclosure of a number of trichomes in a single sheath. This case clearly illustrates, as suggested by Miss Agnes Arber in her excellent monograph on Monocotyledons, that a genus is an evolutionary platform on which species of a different origin are assembled together. In the case of these algæ, three different species of *Anabæna* independently acquired the habit of grouping of numerous trichomes in a single sheath. Obviously we are justified in establishing a new genus, which I call *Anabænothrix*, for the same reasons as we have in establishing *Schizothrix* as a separate genus from *Lyngbya* or *Lyngbya* from *Oscillatoria*. This genus *Anabænothrix* is an expression of a tendency parallel to that of *Schizothrix*, *viz.*, the enclosure of a number of trichomes within a common sheath. This parallelism in evolution is very interesting and shows how the habit of secretion of a common mucus sheath by a number of filaments, developed in different families of Myxophyceæ; by *Schizothrix* in Oscillatoriaceæ and *Anabænothrix* in Nostocaceæ. Members of genus *Lyngbya* are nothing but *Oscillatorias* enclosed in a mucus sheath. Here the individual filaments developed the habit of secreting separate mucus sheaths around themselves, possibly as a protection against drought and desiccation. In *Schizothrix* which marks the next stage, possibly higher in the evolutionary progress we see a number of filaments secreting a common sheath, which means a greater economy of material. This tendency towards collective secretion of a sheath has also expressed itself in *Anabænothrix*, the various species of which developed from different species of *Anabæna*. Though *Anabæna* is structurally on a higher scale than *Oscillatoria* or *Lyngbya*, we find it treading the same evolutionary path as some species of the later two genera of Myxophyceæ. Different species of *Oscillatoria* and *Lyngbya* developed into *Schizothrix* and different species of *Anabæna* into *Anabænothrix*, but both followed

independent roads in reaching the same goal. Following is the possible evolutionary course among these algæ.



We also note similar parallelism in evolution between a dichotomously divided species of *Botrydium* from Madras called *Botrydium divisum*, described by Dr. M. O. P. Iyengar some years ago and *Dichotomosiphon*. The dichotomous nature of the branching of *B. divisum* suggested to Dr. Iyengar some distant connection with *Dichotomosiphon*, a member of Isokontæ. However, these resemblances of form between *Botrydium divisum* Iyengar and *Dichotomosiphon* are purely superficial and comparable to resemblances shown by certain members of Euphorbiaceæ, *e.g.*, *Euphorbia royleana* to *Cereus triangularis*, a member of Cactaceæ. As a result of similar dry and hot environment these plants, phylogenetically far apart, have developed similar physical characteristics like succulence of stem and reduction of leaves. *Dichotomosiphon*, a member of Isokontæ, is characterised by starch metabolism, presence of pyrenoids and presence of carotin and xanthophyll in the same proportion as in higher plants, while *Botrydium*, a member of Heterokontæ, is characterised by absence of pyrenoids coupled with oil metabolism and excess of xanthophyll in its chloroplasts. Differences between Isokontæ and Heterokontæ are regarded as so fundamental that prominent algologists hold that Isokontæ and Heterokontæ had an independent origin among the primitive Flagellates. Most probably the ancestors of *Botrydium* were some dichotomously branched filamentous cœnocytic Heterokontæ, possibly some cœnocytic Tribonemaceæ. Such structure in this hypothetical member of Tribonemaceæ and *Dichotomosiphon* developed quite independently due to a similar response to some unknown environmental conditions and does not mean any phylogenetic relationship between the two, for their differences are more fundamental than their resemblances.

Another striking instance of parallelism in evolution is seen in the case of *Protosiphon*, a globular member of Isokontæ and the common roundish species of *Botrydium*, which belong to Heterokontæ. In this

case the resemblance in external appearance is so great that species of *Botrydium* are often mistaken for those of *Protosiphon* and *vice versa* especially when the chloroplasts have disappeared. The spherical form has been independently evolved in these two algæ to reduce evaporation of water, especially as they subsist on dry mud and they have to economise their water content. These two terrestrial algæ find their parallel in globular Cactaceæ of Mexico and South America.

Species have a tendency to evolve along parallel lines when they are subjected to similar environmental stimuli, *e.g.*, the same degree of temperature, similar hygroscopic conditions and similar soil constituents. This is clearly seen in the case of *Botrydium* and *Protosiphon*. However, in some cases the resemblance may be purely fortuitous and may be due to some unknown changes in the chromomeres of the chromosomes of the nuclei of cells taking place independently in different species of different genera but resulting in similar external physical appearance of the plant.

The discovery of the fact that species have a tendency to evolve along parallel lines has made it abundantly clear that external physical appearance is no index of phylogenetic relationship and we should avoid the fallacy of deducing pedigrees and relationships merely on the occurrence of a few common external characteristics. The realisation of the fact of parallelism in evolution has also influenced our concept of genus. The species of *Anabænothrix* clearly illustrate that genus is an evolutionary platform on which species which have evolved independently from different levels gather together for they have acquired some common characteristics.

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EXPLANATION OF FIGURES.

Anabænothrix cylindrica.

FIG. 1.—Numerous trichomes enclosed in a sheath. (× 360.)

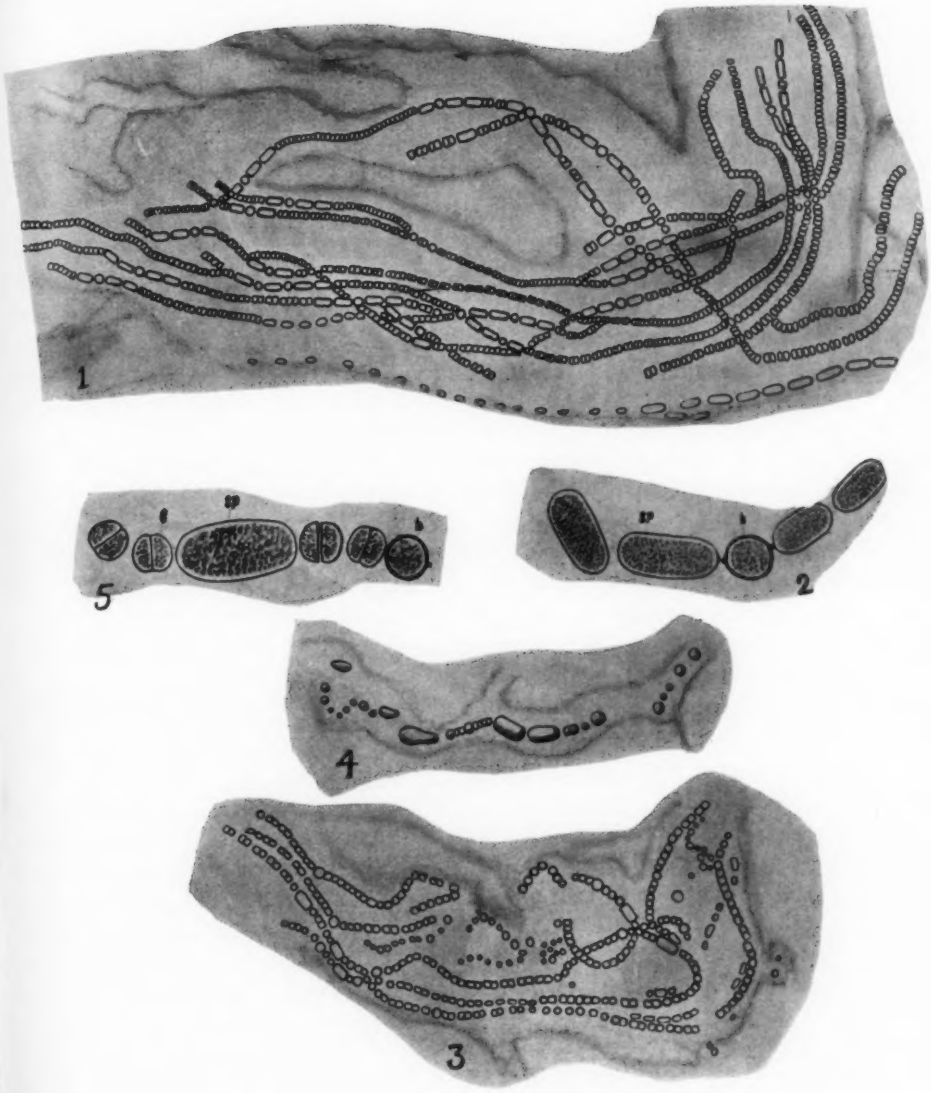
FIG. 2.—*h*, heterocyst; *sp*, spore. (All × 1000.)

Anabænothrix epiphytica.

FIG. 3.—Numerous trichomes enclosed in a sheath. (× 360.)

FIG. 4.—Single trichome enclosed in a sheath. (× 360.)

FIG. 5.—*h*, heterocyst; *sp*, spore; *c*, vegetative cell. (All × 1000.)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STUDIES ON THE MOUTH-PARTS OF MALLOPHAGA INFESTING NORTH-INDIAN BIRDS.

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(Communicated by Dr. M. B. Mirza.)

A. Introduction.

The study of the mouth-parts of Mallophaga is of great value for the Morphologists as well as for those who are engaged in the systematic work on this group of Insects. Snodgrass and Cummings have done a great deal to study the mouth-parts of Mallophaga in a comparative way. Still there are many points to be cleared and a number of modifications to be studied and homologised. The best way to settle the disputed points lies in the comparative study of a large number of forms, from simpler to specialised ones. The observations of the writer of this paper are based on the dissections of as many forms as he could obtain. Still a number of forms remain to be studied in order to clear certain other points with regard to the nature of palpi and the structures associated with pharyngeal glands and sclerite. The present work does not include an important group of Mallophaga infesting the mammals. This is mainly due to the fact that a representative collection of Mammalian Mallophaga is not yet present in the Zoological Museum of the Aligarh Muslim University nor could it be obtained from elsewhere in India.

B. Material and Technique.

The material was obtained from the collection of the Zoological Museum of Aligarh Muslim University, through the courtesy of Dr. M. B. Mirza, Director, Zoological Laboratories. The lice were fixed in Bouin's alcoholic fixative, cleared in 90% and preserved in 80% alcohol. For dissections and mounting, the specimens were treated in caustic potash 5 to 10% according to their size. They were passed through acetic acid, absolute alcohol and were finally transferred to clove-oil for clearing. The dissections were done in clove-oil under a binocular and gave satisfactory results.

C. Description of Mouth-Parts.

(i) *Amblycera*: *Mandibles.*—The mandibles in Ambylcerans are fairly uniform in their features (Fig. 1). Generally in many cases the right mandible

FIG. 1.—Mandibles of Amblycera. *A*, Mandibles of *Myrsidea nigram*; *B*, Mandibles of *Trinoton*; *R*, Right mandible; *L*, Left mandible; *BP*, Basal process; *QP*, Quadrangular process.

is stouter than the left one. They are provided with two or three apices, the tips of which are pointed and densely chitinised. The apices of the right mandible are much more heavily chitinised than those of the left mandible and one apex is blunt while the other is sharply pointed. The cutting surface of each mandible is provided at its base with a protuberance or a short process; that of the right one is smaller and convex anteriorly. The process on the left mandible is larger and concave posteriorly so as to accommodate the process of the right one below it. A number of peculiarly modified and abortive muscle-fibres are attached to the lower end of these processes and the latter are probably of the nature of lacinia mobilis which have fused with the bases of the cutting surfaces of the mandibles.

In general outline the mandibles are roughly triangular, the sides are nearly sub-equal. The condyle is a well-formed rounded projection and it articulates with an anteriorly produced process of gena concave at its distal end; the point of articulation lies just below the origin of maxillary palp at the side of antennal fossa. The ginglymus is more or less an oval groove articulating dorsal to the condyle with a process of clypeus in front of the base of maxillary palp.

The mandibles are provided with two sets of muscles, the one is abductor inserted dorsally near the ginglymus and the other adductor which is inserted near the base of the processes of the cutting surfaces. The latter muscles are stronger than the former.

The mandibles of the Amblycerans are weaker and less modified than those of Ischnocerans. Firstly, because they are removed to the anterior end of the head and secondly, due to their smaller apices. The tips of the apices of one, close outside the tip of the other, opposite the mandible. The working of the mandible is more or less scissor-like for cutting small barbs and barbules along the horizontal-plane.

First Pair of Maxillæ.—The first pair of maxillæ are of fairly generalised features (Fig. 2 B). There is, probably, no room to doubt now that the

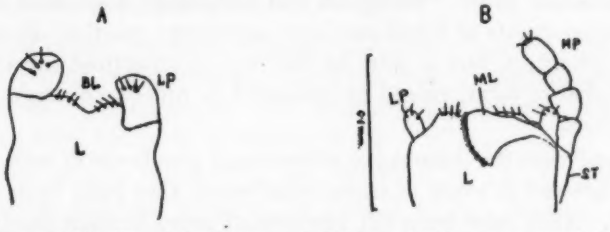

FIG. 2.—Labium and Maxillæ of Amblycera. *A*, Labium of *Trinoton querquedale*; *B*, Labium and Maxilla of *Menopon gallinæ*; *BL*, Bilobed Ligula; *L*, Labium; *LP*, Labial Palp; *ML*, Maxillary Lobe; *MP*, Maxillary Palp; *St*, Stipes of Maxilla.

jointed palpi of Amblycerans which Grosse once assigned to labium, belong to maxillæ. The writer has dissected out the maxilla of a number of forms and observed thinly chitinised strips connecting the bases of the palpi with the stipes in many species of *Læmobothrion* Nitzsch, in *Menopon gallinæ* Linné, and in *Myrsidea nigram* Kellogg and Paine.

The maxillæ are closely approximated to the sides of the labium and since the dissection of the mouth-parts of Amblycerans is slightly difficult, their real structure is often not clearly detected except only in a few forms. The first pair of maxillæ, in a typical form, consists of a jointed palp, a maxillary lobe which has an inner cutting surface beset with short spines and a stipe lying slightly dorsal along the side of the labium. The maxillary lobes are narrow basally, connected with the stipes by thinly chitinised connecting strip. The stipes of the maxillæ are faintly chitinised and closely associated with the sides of the labium. The jointed palpi originate from the stipes and the thin connecting bands can be seen in some cases (Fig. 2 B).

Labium.—In Amblycera labium is distinct and not very greatly modified. In a typical form *cf. Læmobothrion*, we can find a well-marked pre-mentum, a well-developed and broad mentum and a reduced strip of sub-mentum. The pre-mentum carries one jointed palp on either side, which is beset with spines at its tip. The ligula is flat and a number of setæ are borne by it. In *Trinoton querquedulæ* Linne, (Fig. 2 A) the ligula is narrow and bilobed. The labial palp is single jointed and oval in shape. In Colpocephalids the labial palpi are generally very elongated and prominent and beset at their tips with a number of short spines.

Oesophageal Sclerite and Lingual Glands.—The presence of œsophageal sclerite and lingual glands is a special peculiarity of Mallophaga which they share only with Psocids among the Insects. In Mallophaga these structures

are complicated and present a number of modifications specially among the Amblycerans. The sclerite and the associated glands are characters of great taxonomic value. Snodgrass and Cummings have done a great deal to clear many points of detail and have described a number of modifications of these structures, but in spite of this several structures are still doubtful and a number of forms should be examined in order to observe the various modifications.

In Amblycera the œsophageal sclerite and glands are of wide occurrence. They are present in many of those forms where they have been supposed to be absent. They have been left unobserved owing to their thinly chitinised and reduced nature. Cummings' revised list of the distribution of sclerites and glands in Mallophaga needs many alterations because of the separation of a number of forms from the former genera and the creation of new

Genera with sclerite and glands more or less typical	Genera with sclerite and glands modified	Genera with sclerite and glands absent	Genera with sclerite and glands present or absent
Colpocephalum	Boopia	Eureum	Acolpocephalum ?
Læmobothrion	Dennyus	Eomenopon ?	Philandasia ?
Eulæmobothrion	Heterodoxus	Machærilæmus ?	Harrisonia ?
Ricinus	Trinoton		Cummingsia ?
Trochilœctes	Ancistrona		Menopon
Gyropus	Latumcephalum ?		
Somaphantus	Tetrophthalmus		
Myrsidea	Pseudomenopon		
Amyrsidea	Menacanthus		
Neocolpocephalum	Gliricola		
Pseudocolpocephalum*			
Numidicola			
Kurodaia			
Cuculiphilus			
Heleonomus			
Actornithophilus			
Chapiana			
Neumania			

* N. Gen. (in press).

genera and also owing to the fact that recent observations have revealed new data to us. The preceding revised table shows the distribution of sclerites and glands in Amblycera.

Genus *Menopon* possesses typical œsophageal sclerite and glands in nearly all reported cases with the exception of *M. distinctum* and *M. præcursor* recorded by Snodgrass. The other species recorded by the same author have been included in other genera.

Werneck records reduced sclerite in *Gliricola pintoi*. Ewing in his revision and description of the genus *Eureum* Nitzsch does not record the presence of œsophageal sclerite and glands.

A typical and generalised type of sclerite and glands are present in a number of Amblycerans as will be evident from the above table, especially in *Læmobothrion, Menopon, Myrsidea, Colpocephalum* and *Ricinus*. The position of sclerite and glands is more anterior in the head than in Ischnocera. Typically the whole complex consists of a chitinised sclerite with which is associated a frame-work slightly dorsal to it. The frame-work consists of a chitinous bar on either side which unite anteriorly with the sclerite to be produced forward as a pair of anterior cornu. Posteriorly there is a transverse bar joining the side bars which may proceed a short distance behind as posterior cornu. A duct-like tracheal tube comes out from the centre of the sclerite and after a short course bifurcates anteriorly into two diverging arms which bend backward and proceed to the centre of thinly chitinised and roughly spherical, large and paired glands where it branches and sub-branches. Each gland is supported on a chitinous bar which Cumming calls as lateral piece. Over each arm of the duct lies in close opposition an anterior plate which basally abuts against the anterior cornu, its distal end is broad, slightly anterior to the arm of the duct and provided with a number of spines on its inner face (Fig. 3 A).

Description of Special Cases.—Cummings has clearly figured different forms of modification in the genera *Trinoton, Ancistrona, Tetropthalmus, Dennyus*, and *Heterodoxus* which need no recapitulation. Below the writer has mentioned two important cases in which modifications are conspicuous and notable, namely, those of *Menacanthus* Neumann and *Pseudomenopon* Mjöberg.

Menacanthus masudi Qadri. (Fig. 3 C).—The œsophageal sclerite has been recorded to be absent in *Menacanthus* Neumann by Uchida and others. The writer has, however, dissected out a very weakly chitinised and reduced œsophageal sclerite from the above-mentioned form. The œsophageal sclerite resembles to some extent with that of *Tetropthalmus titans* Piaget,

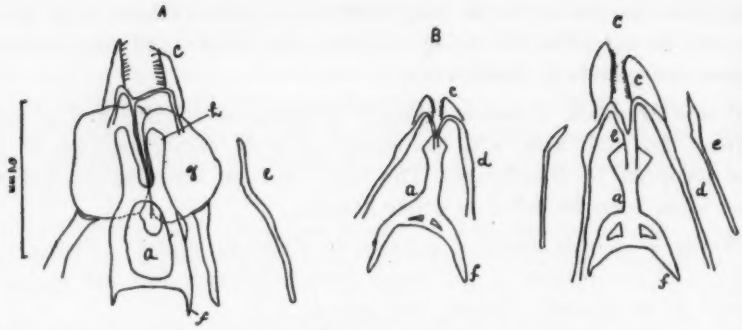

FIG. 3.—Œsophageal sclerite and lingual glands of Amblycera. *A, Menopon gallinæ*; *B, Pseudomenopon poilocephalus*; *C, Menacanthus masudi*; *a*, Œsophageal sclerite; *b*, Fused rami; *c*, Anterior plate; *d*, Lateral piece; *e*, Maxillary fork; *f*, Posterior cornu; *g*, Glands; *h*, Anterior cornu.

described and figured by Cummings. The anterior cornu are wanting. They probably form the short central rod. The rami of the duct are overlain by the paired anterior hypopharyngeal plates and the lateral pieces are comparatively broader and run backward along the sides of the sclerites, diverging posteriorly. There are no glands. The weakly chitinised maxillary forks can be observed lying on the sides of the lateral pieces.

Pseudomenopon poilocephalus Qadri (Fig. 3 B).—The œsophageal sclerite has not been thoroughly described in the genus *Pseudomenopon* anywhere. The writer has observed the sclerite in the above species to be similar in features as in *Menacanthus masudi*. The difference is that the central rod is very short and lateral pieces are stouter and broader. Maxillary forks could not be detected.

The Maxillary Forks.—The so-called maxillary forks have been problematic ever since they have been investigated. The writer has not yet observed the presence of the maxillary forks in any of the Ischnocerans except in *Goniodes dissimilis* N., where they are also mentioned to exist by Cummings. The homology and function of these maxillary forks have not yet been ascertained, and various divergent views have been mentioned by Cummings. To the writer they appear to be vestigeal sclerites of the hypopharynx. They gradually atrophy and are missing among specialised forms of Amblycerans, *cf. Colpocephalum* and others, and finally almost wanting among Ischnocerans.

Another structure associated with these sclerite and glands is a median thinly chitinised plate posterior to the glands between the two lateral pieces. The writer has observed in *Læmobothrion* sp. ? (Fig. 9 A) the opening of the common salivary duct on it. It is probably the basal part of hypopharynx,

whereas the lingual glands and œsophageal sclerite are the modifications of the rest of the hypopharynx and its associated structures.

(ii) *Ischnocera.*—Mandibles are much more strongly built in *Ischnocerans* than in Amblycerans. They are also remarkable for the fact that they are inserted at right angles to the head, the condyle being posterior and ginglymus being anterior. A further marked difference lies in the position of the mandibles in the head of Ischnocerans. In Amblycera they lie near the anterior end of the head but in Ischnocera they retrace far back nearly to the centre of the head. The mandibles of Ischnocera (Fig. 4) are more or less tri-

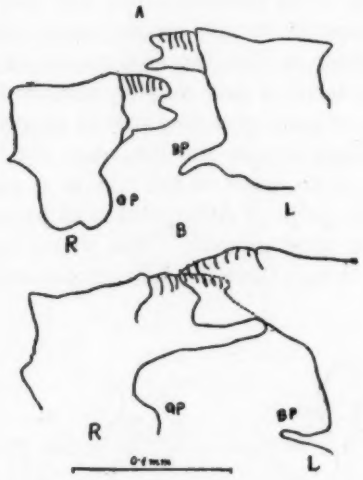

FIG. 4.—Mandibles of Ischnocera. *A, Lipeurus caponæ*; *B, Philopterus rotundatus*; *BP*, Basal process; *QP*, Quadrangular process; *R*, Right mandible; *L*, Left mandible.

angular. They are in many cases provided with two apices, those of the right mandibles being slightly more developed. The apices of the mandibles in most of the cases are dorsally striated and produced inward. Among the Ischnocerans of Indian Birds the writer has found that the mandibular apices are not striated in *Goniodes dissimilis* N. only. This fact, together with other features of the mouth-parts, to be described later on, suggests that forms belonging to *Goniodes* N. are primitive among the Ischnocerans.

Towards the base of the cutting surface the left mandible is provided with a long and posteriorly concave process—the basal process. In the right mandible at the corresponding position is not a very prominent protuberance, the quadrangular process. In addition to these processes

there may be additional processes in the mandibles in the middle of the cutting surfaces which project and are called Auricularian processes.

The working of the mandibles is peculiar in Ischnocera. The tips of the apices are bifid and fit outside each other. The basal process of the left mandible does not come over the quadrangular process of the right and in this way cutting surfaces of the mandible do not completely cross each other. It is only the apex which performs most of the cutting work. The bifid tips of the apices fit outside each other and nibble off the barbes and barbules of the feathers of birds.

1*st Pair of Maxillæ*.—The problem of the first pair of maxillæ and their palpi has been a point of dispute among many workers on this group. Shipley in his description of sketches of *Goniodes tetraonis* Denny marks the labial palpi as the lobes of first maxilla and small chitinised processes on the ligula of labium as palpi of second pair of maxillæ; on the other hand, Cummings rejects Shipley's view and describes the labial palpi as paraglossæ and the chitinous processes on the labium as chitinous nodules. He also describes the labial palpi of Amblycerans as paraglossæ, a view which is highly disputable on good grounds. The writer has observed in many Ischnocerans (see Fig. 5) that there is a lobe supported by a chitinous frame-

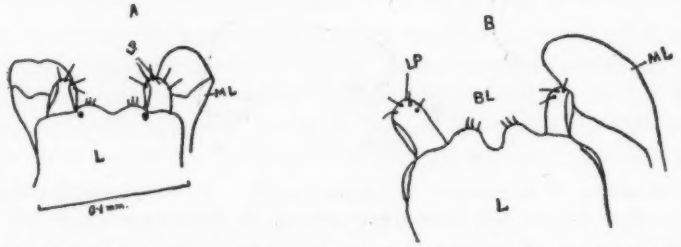

Fig. 5.—Maxillæ and Labium of Ischnocera. *A, Columbicola ewingi*; *B, Philopterus rotundatus*; *BL*, Bilobed Ligula; *L*, Labium; *LP*, Labial Palp; *ML*, Lobe of Maxilla.

work situated on each side of the labium and slightly dorsal to the latter. These structures from their form and position appear to represent the maxillæ in which the maxillary lobes and palpi are highly reduced. In *Goniodes dissimilis* N., the maxillæ are more prominent than in others. They are very well formed in *Columbicola ewingi* Qadri.

2*nd Pair of Maxillæ*.—The second pair of maxillæ or labium is more or less similar to that found in Amblycerans (Figs. 5 and 6). It consists of a broad thinly chitinised mentum and small pre-mentum which carries ligula and palpi. It is about the ligula and the palpi that a great difference of

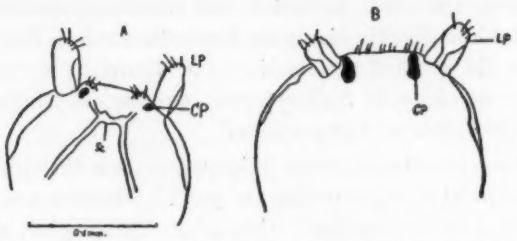

FIG. 6.—Labium of Ischnocera. *A, Esthiopterum secretarium*; *B, Goniodes dissimilis*; *CP*, Chitinous process; *LP*, Labial Palp; *Sc*, Sclerite.

opinion exists. The ligula is either flat or slightly bilobed in the middle. Below each palp there is a chitinous nodule or process very prominent and well-developed in *Goniodes* N., specially in *G. tetraonis* Denny. In *G. falcicornis* N., Cummings has shown it to be very small and describes it as chitinous nodule. In *G. dissimilis* N., (Fig. 7) they are mid-way between

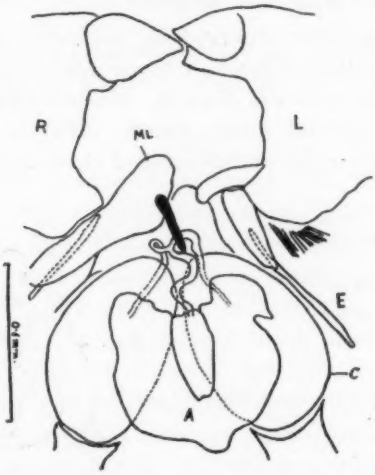

FIG. 7.—*Goniodes dissimilis*—Mouth-parts. *A*, Œsophageal sclerite; *C*, Lingual gland; *E*, Maxillary fork; *L*, Left mandible; *R*, Right mandible; *ML*, Lobe of maxilla.

G. tetraonis D., and *G. falcicornis* N. The writer has observed similar nodules smaller than those of *Goniodes* to exist in a number of forms.

A characteristic feature of the labium of the Ischnocera is the presence of a sclerite on the dorsal surface of it. The sclerite consists of a transverse band just below the ligula. It has got a pair of limbs at each end. One of the pairs is anterior and runs towards the base of the labial palpi into the

chitinous process, the other posterior runs backward towards the posterior margin of the labium slightly diverging from the median line. It is peculiar that the writer did not find the sclerite in *G. dissimilis* N., though it is clear in many forms specially in *Esthiopterum secretarium* Giebel, *Columbicola ewingi* Qadri and *Lipeurus caponœ* Linné.

As it has been mentioned above Shipley describes the chitinous processes below the labial palpi as representing the palpi of labium and the labial palpi as lobes of first pair of maxillæ. Cummings homologises the labial palpi with those of the Amblycerans but describes them in both as paraglossæ. In my opinion the paraglossæ of Cummings and first maxillæ of Shipley are same and homologous and belong to labium or second pair of maxillæ and are homologous with the labial palpi of Amblycerans. This view specially in *Amblycera* is held by a number of old and modern workers, *e.g.*, Ewing, Ferris and others. The palpi of 2nd pair of maxillæ of Shipley and chitinous nodules of Cummings are specially chitinised lobes developed in connection with ligula. The ligula, as already mentioned above, is generally flat, sometimes bilobed and beset with erect spines.

The œsophageal sclerites observed by the writer in the genera *Degeeriella* Neumann, *Philopterus* Nitzsch, *Columbicola* Ewing, *Lipeurus* Nitzsch, *Esthiopterum* Harrison, *Goniodes* Nitzsch, *Neophilopterus* Cummings and *Eustrigiphilus* Ewing, are not much widely different from their typical form (Fig. 8). The only great modification of these structures was observed

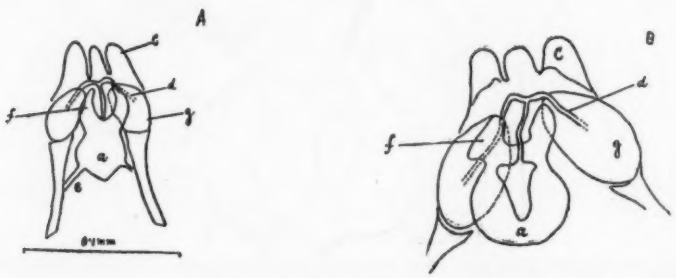

FIG. 8.—Œsophageal sclerite and lingual glands of Ischnocera. *A*, *Columbicola ewingi*; *B*, *Degeeriella subcuspidatus*; *a*, Œsophageal sclerite; *C*, Anterior plate; *d*, Duct; *e*, Posterior cornu; *f*, Anterior cornu; *g*, Lingual gland.

in *Ibedœcus* Cummings. The anterior cornu are present in nearly all the forms, the posterior cornu are present in some forms, while absent in others. The lingual glands are hard, oval and thinly chitinised. The posterior median lobe of hypopharynx on which the salivary duct opens overlies the base of the labium and is situated between the two lateral pieces. It can be

observed in *P. rotundatus* Piaget (Fig. 9 A). Maxillary forks were not detected in any case except in *G. dissimilis* N.

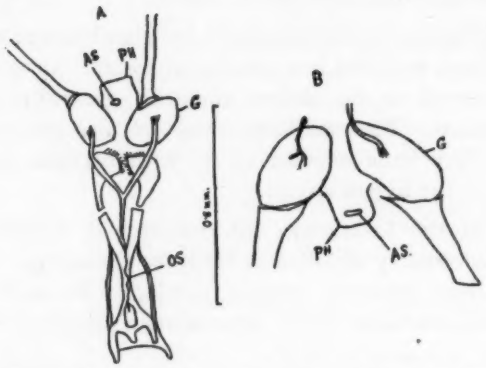

FIG. 9.—Hypopharynx and lingual glands. *A, Læmobothrion* sp.; *B, Philopterus rotundatus*; *AS*, Aperture of salivary duct; *G*, Lingual gland; *OS*, Œsophageal sclerite; *PH*, Posterior lobe of hypopharynx.

The greatly modified pharyngeal sclerite described by Cummings in *Ibedæcus flavees* Cummings has its corresponding structures in *I. robustum* Qadri.

D. Summary.

1. The mandibles of Ischnocera, apart from their other well-known features, are stouter than those of Amblycera. Their apices are striated in most of the cases in Ischnocerans, an important exception being *Goniodes* N.

2. First pair of maxillæ are present in Amblycera with a jointed palpi, a maxillary lobe beset with spines at its inner face, and a stipe closely applied to the labium. The connecting bands between palpi and stipes can be observed in a number of forms specially in *Læmobothrion* N., in *Menopon gallinæ* Linné and *Colpocephalum semicirctum* Rudow.

3. First pair of maxillæ in Ischnocera are represented by reduced lobes present on the sides of the labium. Their stipes with chitinous frame-work can be easily observed in *Goniodes dissimilis* N., *Columbicola ewingi* Qadri, and *Esthiopterum secretarium* Giebel.

4. The labium or second pair of maxillæ in Amblycera consist of a reduced sub-mentum, a broad and thinly chitinised mentum and a pre-mentum with a reduced and flat or bilobed ligula. One or two jointed labial palpi are present.

5. Labium of Ischnocera has its corresponding parts in that of Amblycera. One jointed labial palpi are present in nearly most of the forms. The

so-called chitinous nodules of Cummings or labial palpi of Shipley described in *Goniodes* N., and found in others as well, are short chitinous processes developed in connection with the ligula.

6. The œsophageal sclerite and associated glands are of wide occurrence in Amblycera, though modified in a number of forms. In most of the cases when they are believed to be absent they are generally highly reduced and weakly chitinised. The maxillary forks are also not very uncommon in Amblycera. They have nothing to do with maxillæ and seem to be vestigeal sclerites of the hypopharynx.

7. In most of the Ischnocera the œsophageal sclerite is strongly chitinised. It is remarkably modified in *Ibedœcus* Cummings. The *maxillary* forks have so far been detected only in *Goniodes dissimilis* which along with other features mentioned above, represents a primitive form among the Ischnocerans.

8. A posterior median lobe of hypopharynx can be observed in *Læmobothrion* sp. ? and *Philopterus rotundatus* Piaget, between the two lateral pieces supporting the lingual glands. On this opens the common duct of the salivary glands.

E. Conclusion.

The anatomy of mouth-parts in Mallophaga is still a disputed problem. Snodgrass, Cummings and others have done a good deal to explain various structures found therein. There is, however, still some dispute over certain structures with labium, maxillæ and œsophageal sclerite. Apart from this, various modifications in a number of forms have got to be homologised. The homology of the œsophageal sclerite and its associated glands is far from being decided. The writer agrees with Cummings, Mjöberg and Grosse to call these structures as a compound hypopharynx. The lingual glands are most probably greatly modified superlingue as Enderlein thinks them to be. The maxillary forks, as the writer has mentioned above, are vestigeal sclerites of hypopharynx, and not superlingue or maxillulæ. The above-mentioned opinion is, however, open to question until more work is done on the anatomy and specially on the mymphal stages of various biting-lice.

The writer takes this opportunity to offer his sincere thanks to Dr. M. B. Mirza for his keen interest and guidance during the preparation on this work.

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at the Agricultural Research Institute,

Coimbatore.

A NOTE ON THE BREEDING OF SUGARCANE VARIETIES RESISTANT TO MOSAIC.

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The evolving of disease resistant varieties is now an important item in the programme of practically all plant breeding stations. In the sugarcane, the plant breeder has had to contend with diseases from the very first. It will be of interest to those not very familiar with the history of sugarcane breeding to know that it was the serious outbreak of "sereh" in Java in the eighties that called attention to the desirability of producing new varieties by hybridisation which might be resistant to this disease. Kobus came to India in 1890 in search of varieties resistant to "sereh", with a view to utilising them later for breeding purposes in Java. This enterprise of his was rewarded by the variety Chunnee (*Saccharum barberi*, Jeswiet), which not only proved itself resistant to "sereh" but also gave rise, on breeding, to seedlings that were resistant to the disease. Thus began the first series of P. O. J. canes which were crosses between Black Cheribon and Chunnee.

Latterly, the disease, which has claimed the serious attention of sugarcane pathologists and breeders, is mosaic which during recent years assumed such large proportions in certain countries. Mosaic appears to have been first noticed in Java by Musschenbroek in 1892, though it was called by the name 'yellow stripe'. Valuable contributions as to the real nature of mosaic were later made by Wilbrink (1910, 1922), Lyon (1917, 1921), Stevenson (1917), and others. It was in 1919–20 that Brandes was able, successfully, to transmit mosaic under controlled conditions and demonstrate that *Aphis maidis* was the insect vector. As is well known *Aphis maidis* is an insect which feeds commonly on maize, Sorghum and various other grasses. In the words of Earle (1928), "it is a most remarkable fact that so serious a disease can be spread so rapidly by an insect that does not normally feed on the cane plant."

Thanks again to Brandes (1925) and various other workers, the behaviour of the four species of *Saccharum* to mosaic is now known with a fair amount of definiteness. The majority of the recent commercial seedling varieties of sugarcane contain the blood of two or more of the following species of *Saccharum* :—(i) *S. officinarum*, to which belong the thick-stemmed or 'noble' canes like Pundia and Poovan in India and Black Cheribon and Lahaina in other countries. (ii) *S. sinense*, which includes the Pansahi group of Barber, *e.g.*, Uba, etc. (iii) *S. barberi* and (iv) *S. spontaneum* comprising the North Indian and the wild reed-like canes respectively. As regards the first group, *i.e.*, the thick canes (*S. officinarum*) "all of the varieties, so far studied, are susceptible to mosaic and most of them are severely injured when they become infected". Certain of the members of the second group (*S. sinense*) are definitely immune and some are susceptible but "the susceptible ones are remarkably tolerant". The North Indian canes (*S. barberi*) are very susceptible to mosaic but very little injured by it. The last group (*S. spontaneum*) contains several forms or varieties which are characterised by complete immunity to mosaic. Certain of the Papuan wild canes have been put under a new species which has been provisionally named *S. robustum* (Jeswiet). Brandes has reported that one of the varieties belonging to this species—28 N.G. 251—readily became infected with mosaic when exposed to natural infection in Porto Rico (Brandes, 1931).

Excepting the canes of the Pansahi group (*S. sinense*), which are generally infertile under Coimbatore conditions, the other three species have been crossed extensively and seedlings are now available which, in their resistance to mosaic, represent all gradations from susceptible to immune types. We may now consider the behaviour of certain of these interspecific hybrids. Kassoer, which is supposed to be a cross between Black Cheribon and Glagah (the Java form of *S. spontaneum*), is not only itself immune to mosaic but all of its selfed seedlings examined by Brandes (1925) proved immune without exception. From the Co. canes the example of a cross between a thick cane and *S. spontaneum*, is the seedling variety Co. 205, which is a cross between Vellai (a cane resembling Lahaina) and the Coimbatore form of *S. spontaneum*. Co. 205 has been found to be susceptible to mosaic in North India (McRae and Subramaniam, 1928) while in the experiments at Coimbatore (Sundararaman, 1932–33) it has been found to be highly resistant. Co. 229 is a selfed seedling of Co. 205, but no data are available as to its percentage of infection. Co. 317 and Co. 318 are selfed seedlings of Co. 229. Of these, Co. 317 gets about 70 per cent. infection while Co. 318 has, so far, remained free.

The seedlings of the crosses between *S. officinarum* and the North Indian cane Chunnee, which were raised in Java have, according to Brandes (1925), proved susceptible but tolerant to mosaic. Instances are, P. O. J. 36, P. O. J. 213 and P. O. J. 234. At Coimbatore, P. O. J. 213 was crossed with certain North Indian canes. The following are the seedlings of these crosses :—Co. 213, Co. 230, Co. 231, Co. 232 and Co. 233. Of these, Co. 213 and Co. 232 are highly susceptible while the percentage of infection in the other three varieties is not known. P. O. J. 213 was also crossed with Co. 205 and one of the seedlings of this cross, Co. 244, has proved resistant to mosaic at Coimbatore, while it is reported to take the disease in North India. Co. 214 is an interesting case. It is a cross between Striped Mauritius (a variety of *S. officinarum*, and susceptible to mosaic) and a selected seedling of Saretha × *S. spontaneum*. The ultimate parentage of Co. 214 is, therefore, Str. Mauritius × Saretha × *S. spontaneum*. Co. 214 has proved immune to mosaic. Co. 335, a selfed seedling of Co. 214, is also immune to mosaic (Sundararaman, 1933–34). During recent years in the work at Coimbatore for the breeding of thick type of canes for Tropical India as also for the semi-irrigated conditions of North India, the varieties P. O. J. 2725, P. O. J. 2727, P. O. J. 2878 have been extensively employed as female parents. Co. 408 is a seedling of P. O. J. 2725 × Co. 243, and is reported to get only 13·5 per cent. mosaic at Coimbatore, while Co. 412 and Co. 421 whose ovule parent is P. O. J. 2878 show 10·5 and 28·3 per cent. of mosaic infection respectively. Co. 419 is a seedling of the cross P. O. J. 2878 × Co. 290 and has shown 43 per cent. infection. Co. 411, a seedling of the cross P. O. J. 2727 × P. O. J. 2878, has shown 4 per cent. mosaic. It may be mentioned that P. O. J. 2725 is considered to be fairly resistant to mosaic and P. O. J. 2727, though not so resistant as P. O. J. 2725, is known to take the disease very rarely, while P. O. J. 2878 is highly resistant.

Brandes (1925) has shown that " the quality of immunity is proportional to the amount of *S. spontaneum* blood contained in the seedling. When this is increasingly diluted an increasing number of susceptible seedlings are to be expected in the progenies". The above observation of Brandes is corroborated in a number of cases by the experience at Coimbatore also. Co. 214 has only 25 per cent. *S. spontaneum* blood in it and is immune to mosaic, while the seedlings Co. 223, Co. 241 and Co. 287, which have no *S. spontaneum* blood in them have proved susceptible. The seedlings Co. 317 and Co. 318 bring out the fact that canes of the same parentage having a certain amount of *S. spontaneum* blood in them, may yet vary in their resistance to mosaic and it is for the breeder to select only such as

are resistant to the disease. Co. 313 contains 12·5 per cent. *S. spontaneum* blood and though highly susceptible is tolerant. The usefulness of the introduction of *S. spontaneum* blood is therefore apparent. The sugar industry in India owes much to the foresight of Dr. Barber, who deliberately used *S. spontaneum* in his breeding work for introducing hardiness and vigour into the seedlings. The seedlings containing *S. spontaneum* blood have, besides being vigorous and hardy, the additional advantage of resistance to mosaic. A similar case is the utilisation of Chunnee in Java. Kobus used it in order to fight the " sereh " disease, but Chunnee, in addition to " sereh " resistance, passed on to its seedlings the quality of resistance to cold and frost. Certain of the seedlings of this cross, *viz.*, P. O. J. 36, P. O. J. 213 and P. O. J. 234, have made themselves useful to the sugar industry in certain other countries, though in Java itself these qualities of resistance to cold and frost would hardly be needed to play their part.

We may now refer to the inter-genetic crosses. Broune (1934), working in Florida, has reported that none of the hybrids between the cross P. O. J. 2725 × Sorghum made in Florida, have been found to be susceptible to mosaic. On the other hand, certain of the seedlings of P. O. J. 2725 at Coimbatore have proved susceptible to mosaic, *viz.*, Co. 354 and Co. 515. It may be mentioned; however, that Co. 355, a seedling of the same cross, has proved resistant to this disease. Though not a strictly parallel case but an interesting one in this connection is "the case of inheritance of resistance to aphis observed by Gernert in F_1 hybrids between teosinte and corn. Both corn root-aphis, *Aphis maidiradicis*, and the corn plantaphis, *A. maidis*, were involved and both the teosinte and the hybrids were completely resistant while the corn was badly infested." (Babcock and Clausen, 1927.)

One handicap in the practical aspect of breeding disease resistant varieties is, that resistance is likely to vary geographically. Two instances of this type have already been mentioned, *viz.*, the differential behaviour of Co. 205 and Co. 244 with regard to mosaic in North and South India. Another instance is the sugarcane variety Pundia, a standard cane in the Bombay-Deccan where it is not known to suffer from mosaic while at Coimbatore it has shown 25 per cent. infection. Co. 290 may also be added to this list, since in North India and in certain foreign countries it is reported to be highly resistant, while in the Coimbatore experiments it has shown about 70 per cent. infection (Sundararaman, 1930–31). The case of Pundia is somewhat unique. It belongs to *S. officinarum* and would, therefore, be expected to suffer from the disease, but no case of mosaic has been reported on Pundia in the Bombay Presidency though the disease has been noticed on certain other canes in that Presidency. As is well known, the geographical

variation in resistance is due, in certain cases, to the existence of physiologic races of the causal agent. In sugarcane also it has recently been found by Summers (1934) that there are four distinct types of mosaic symptoms on the sugarcane varieties C. P. 28/60 and Co. 281 in Louisiana. All the four types have persisted without apparent change through successive vegetative propagations of the infected plants, and all the four were perpetuated readily by transfer of inoculum from diseased to healthy plants of the same variety. These observations are of more than ordinary interest, as one of the types is very destructive to cane varieties formerly regarded as resistant to mosaic. (*Facts About Sugar* 1934, p. 408.)

Correlations between morphological characters and disease resistance, if substantiated, will undoubtedly be a valuable aid to the plant breeder in selecting types resistant to the disease. Venkatraman and Thomas (1928) expressed the opinion that immunity of such varieties as Kassoer might be due to the possession or inheritance of bristles which protect the stomata and the surrounding region from the attack of insect vector. Two of us (Dutt and Krishnaswami) have recently examined over a dozen varieties in this connection. Camera lucida drawings of the lower epidermis of 10 varieties are shown in Fig. 1. It will be seen that the number of bristles in Black Cheribon and Vellai is few. In fact, in the preliminary count of bristles per unit area, the number of bristles was few in all the *S. officinarum* varieties that were examined. Co. 214 was found to have about the same number of bristles per unit area as Vellai and Black Cheribon and yet Vellai is highly susceptible, while Co. 214 is highly resistant. Same is the case as regards P. O. J. 2878. The number of bristles per unit area in Co. 205, which has been found to suffer from mosaic in North India, is far more than in Co. 214 which has proved highly resistant wherever it has been grown. A reference to Fig. 1 will also show that stomata often lie unprotected even in Kassoer and Glagah, while a large majority of them are entirely exposed in Co. 214 and P. O. J. 2878. This preliminary examination, therefore, indicates that there is perhaps no positive correlation between the high number of bristles and disease resistance, nor in the protection supposed to be afforded by the bristles to the stomata.

In this brief note an idea has been given of certain aspects of the question of mosaic resistance in sugarcane varieties. It may be said that the ultimate solution lies in the breeding of varieties that would be resistant to the various cane diseases and as in the sugarcane, a range of varieties and species is available whose behaviour with respect to diseases is known and which fortunately cross freely *inter se*, the prospect for the sugarcane breeder is not discouraging.

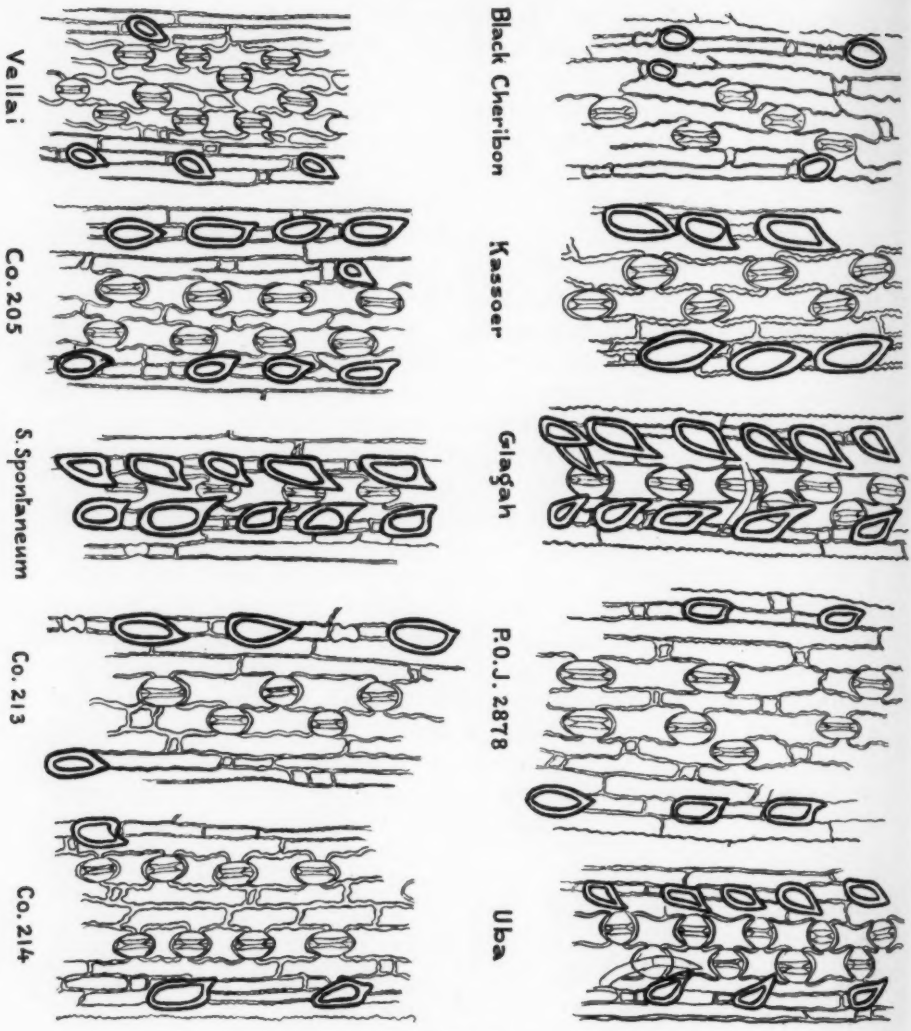

FIG. 1.
Bristles on the Lower Epidermis of certain Sugarcane Varieties.

Summary.

The behaviour of certain Coimbatore sugarcane varieties with reference to the mosaic disease has been discussed, showing that those containing *Saccharum spontaneum* blood are generally resistant or at least tolerant.

Preliminary data regarding the supposed correlation between bristles and mosaic resistance have been presented, which indicate that at least in certain cases there appears to be no positive correlation between the high number of bristles and disease resistance, nor in the protection supposed to be afforded by the bristles to the stomata.

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EXPLANATION OF FIG. 1.

The magnification of all original drawings, × 390.
The drawings have been reduced to ½ in reproduction.

Black Cheribon: A variety of *Saccharum officinarum.*
Kassoer: Supposed to be a seedling of Black Cheribon × Glagah.
Glagah: The Java form of *S. spontaneum.*
P.O.J. 2878: The famous Java seedling variety.
Uba: A variety of *S. sinense.*
Vellai: A variety of *S. officinarum.*
Co. 205: A Coimbatore seedling of Vellai × *S. spontaneum.*
Saccharum spontaneum: The Coimbatore form of *S. spontaneum.*
Co. 213: A Coimbatore seedling variety.
Co. 214: A Coimbatore seedling variety.

NATURAL AND INDUCED RESISTANCE TO SHOOT-ROT IN THE COCONUT.*

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Introduction.

Long before there was any exact knowledge concerning the origin and nature of the dissimilarity exhibited by related individuals exposed to the same environmental conditions, such dissimilarity was clearly recognised. It was further recognised that related individuals differed among themselves in respect of their susceptibility to disease.

Using the term disease in unrestricted sense, so as to include functional disorders the causes of which are obscure or difficult of analysis, the resistance or immunity to disease can be conveniently classified as natural and induced or acquired.

Apart from the natural immunity due to genetic constitution, it is well known that resistance can be, at least in some cases, induced by altering the cultural conditions under which the susceptible forms grow. In this brief paper we wish to record some observations regarding the natural and induced or acquired immunity.

Observations.

About 1921, the shoot-rot of the coconut seedlings was first noticed at the Agricultural Research Station, Pilicode (District : South Kanara), and in subsequent years it has been noticed in the neighbouring gardens. Unlike "Leaf-rot" which is known in several parts of the West Coast, the disease is confined to the tender central shoot, consisting of tender leaves. Even though in the later stages the disease resembles " bud-rot", it can be distinguished in earlier stages from the latter in that the rotting first commences at the tip of the shoot, while in bud-rot the infection is at the base of the bud. The affected parts may in advanced stages emit a foul odour.

After a prolonged examination of the infected material in different stages of development, Sundararaman (1929) isolated three species of organisms,

* Paper No. 11 of the Oil-Seeds Section of the Madras Agricultural Department.

viz., *Glœosporium* sp., *Fusarium* sp., and *B. Coli*, from the diseased material. From inoculation experiments he has concluded that *Glœosporium* sp. is the causative organism.

Sundararaman and Krishanswami (1933) state that the fungus causing the disease is a weak parasite which ceases to do damage in normal weather. During the heavy monsoon rains of July to September, the attack is severe. As a preventive, the trees are regularly sprayed with Bordeaux mixture. That the disease is an important one is evident from the fact that out of about 1,700 palms of different ages, on an average, 470 palms are affected and about 60 of them die due to the disease.

TABLE I.

Incidence of Shoot-Rot. (No. of Palms.)

Year	Infections*	Deaths
1930	462	25
1931	498	168
1932	551	52
1933	516	28
1934	339	32
Average	473	61

*Out of a total of 1731 palms.

Age of the Palm and Susceptibility to the Disease.

The disease is less common in very young seedlings and mature trees. One and two year old seedlings are not susceptible to the disease. Trees over thirteen years of age are rarely infected with shoot-rot. The palms of three to nine years are most susceptible, *vide* Table II.

TABLE II.

Age of the Palm and Susceptibility to Disease.

Year	Percentage of infections among palms of different ages										
	3 yrs.	4 yrs.	5 yrs.	6 yrs.	7 yrs.	8 yrs.	9 yrs.	10 yrs.	11 yrs.	12 yrs.	13 yrs.
1925 ..	3.0	..	..	10.1	12.5	..	..	..	..	..	..
1926 ..	38.0	..	..	..	10.0	13.1	..	..	..	..	..
1927 ..	50.4	51.3	..	..	..	18.7	15.5	..	..	..	..
1928 ..	23.8	48.0	43.5	3.0	..	..	..	2.3	..	..	..
1929 ..	27.3	64.6	75.2	53.0	..	..	..	12.6	23.5	..	..
1930 ..	18.6	30.2	46.2	60.8	46.9	..	..	..	5.5	8.6	..
1931 ..	15.3	44.0	32.9	38.7	62.4	48.9	..	..	..	2.6	11.9

Natural Resistance to the Disease.

Twenty-six varieties of the coconut from different parts of the world are grown at Pilicode for economic work. The appearance of the disease in these varieties affords an opportunity to study the varietal resistance to the disease. No variety is completely immune to shoot-rot. The incidence of disease in each variety was recorded, and the observations were confined to three to nine year old palms. The mean percentages of infections (*vide* Table III) show that the variety from the Philippines is the least susceptible. Among the Indian varieties, the variety from Omalur is considered to be the best since only 50 per cent. of the palms are affected with shoot-rot. The varieties from Mysore and Chowghat with over 85 per cent. of infection are most susceptible.

TABLE III.

Varietal Resistance to Shoot-Rot.

Variety from	Mean†	Variety from	Mean†
Mysore ..	87·7	Komarapalayam ..	67·8
Chowghat ..	85·0	Kulitalai ..	66·7
Godavari ..	84·5	Kasaragod, I.R. ..	65·9
Kasaragod, R. ..	83·7	Laccadives, C. ..	64·9
Straits Settlements ..	79·5	Celrospuram ..	63·4
Gudiyattam ..	78·5	Badagara ..	63·2
Indupalli ..	78·0	Bombay ..	60·1
Pollachi ..	74·7	Tanjore ..	58·2
Tikkoti ..	74·7	Kasaragod, R. L. ..	57·4
Fiji ..	71·2	Indupalli, O. B. ..	55·9
Ediakanad ..	69·3	Andamans ..	55·2
Laccadive, O. ..	68·6	Omalur ..	49·7
Bengal ..	67·9	Philippines ..	37·4

† Mean percentages of infections for palms of three to nine years (inclusive) ages.

Induced Resistance.

From the observations in the preliminary manurial experimental plots, it appeared that the plots receiving nitrogen, nitrogen *plus* phosphate and the control plots were more subject to the disease than the plots receiving N + P + K or N + K or K alone. In order to obtain conclusive data two treatments and a control were laid down and replicated three times. The treatments were : (*a*) the application of Potassium sulphate at the rate of 3 lb. per tree, (*b*) the application of lime at the rate of 20 lb. per tree.

TABLE IV.

Incidence of Shoot-Rot in Manurial Plots.

Treatment	Percentage of infections in		
	1933	1934	1935
K_2O ..	56·1	26·43	2·22
Lime ..	48·7	29·3	35·82
No manure ..	96·2	36·73	47·82

On the whole the incidence of disease was considerably less in the plots receiving potassium sulphate. The lime may also, to a certain extent, help in inducing resistance to the disease.

In an experiment designed to test the efficiency of the different systems of planting the seedlings, it was found that the incidence of disease was lowest in the palms which were planted at a depth of three feet.

The incidence of attack in the surface-planted palms was double of that in the palms planted at a depth of three feet.

TABLE V.

Depth of Planting of Seedling and Incidence of Infection.

Deapth of planting	No. of infections in						
	1926	1927	1928	1929	1930	1931	Mean
Surface‡ ..	2	11	15	17	16	14	12·5
One foot ..	..	4	15	16	7	10	8·7
Two feet ..	1	5	7	15	11	16	9·7
Three feet ..	4	4	6	10	7	9	6·7

‡ There were 30 palms in each of the plots.

Discussion.

We have seen that resistance can be artificially induced by deep-planting and through the application of potash fertilisers. It has also been mentioned that some varieties are more resistant than the others. If we conceive disease, defect, derangement, as marked deviations from the standard of normality of the species and race under consideration, and as states of the individual at the limit of its accommodation, then the nature of acquired and natural resistance should be similar. The hereditary constitution of the individual can be regarded as an unbolting mechanism, the genetic factors coming into action serially and releasing physiological agents which specifically affect developmental processes. Inborn errors of metabolism and biochemical disharmonies which predispose to the development of diseased states are often rectified by suitable changes in the cultivation of a crop. That the defect due to the absence of a gene for immunity can be corrected by changes in the nutritive conditions suggests that the difference between acquired and natural resistance is narrow.

Through nutritional alterations it is possible to maintain the general efficiency of the plant (to avoid disease generally) and to increase the protoplasmic resistance so as to disarm the pathogene after entrance. It has not, however, been possible through nutritional changes to prevent the entrance of the attacking organism. Pubescence or waxy excretions prevent sometimes the entrance of the disease, the number of the stomata or the arrangement of cells beneath them may influence fungus infection. These conditions have not been yet changed by modifications of the nutrition of the plant.

Since a number of diseases are inherited in simple Mendelian manner, the study of the exact manner in which the genes affect the metabolism of the plant offers a vast field for the physiologist to explore the true nature of the phenomenon which we ultimately know as disease resistance.

Summary.

Observations on the varieties of the coconut collected from the different parts of the world and grown at the Agricultural Research Station, Pilicode (District : South Kanara), showed that the precentage of infections varied from 37—the lowest in a variety from the Philippines—to 87 in a variety from Mysore.

The disease occurs largely among the palms of three to nine years of age, and thereafter the trees are generally less susceptible to the disease. The susceptibility to the disease is more pronounced in the trees planted on the surface, than in the trees planted at a depth of three feet. The incidence of

disease is considerably reduced when potassium sulphate is applied to the soil. The bearing of these observations on the subject of natural and induced resistance to the disease is discussed.

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A PRELIMINARY NOTE ON THE MODE OF INHERITANCE OF REACTION TO WILT IN *CICER ARIETINUM*.

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One of the handicaps for the successful cultivation of Bengal gram (*Cicer arietinum*) in the Coimbatore district is the wilting of a fairly large number of plants during their growth (*vide* Plate XXI). Counts of such plants made in the crops raised at the Cotton Breeding Station, Coimbatore, during 1930–35 showed that the annual loss on this account varied from 14 to 28%. These deaths were traced by Narasimhan (1928)* to a fungus of the *Fusarium* group.

Among the many strains isolated at the Cotton Breeding Station, Coimbatore, primarily for variation in yield, two, *viz.*, Nos. 19 and 468, manifested consistently marked differences in their reaction to wilt. Their behaviour during the past several years are given in Table I.

Table I.

Annual variation in the percentage of mortality.

Year	Strain No. 19	Strain No. 468
1931—32 ..	42·0	1·5
1932—33 ..	43·0	2·0
1933—34 .	43·0	0·4
1934—35 ..	48·0	6·0
1935—36 ..	36·0	7·0

* R. Narasimhan, "A preliminary note on a *Fusarium* parasite on Bengal gram (*Cicer arietinum*), *Mad. Agri. Dept. Year-book*, 1928.

Since these strains were not grown on the same site every year and since no precaution was taken to see by artificial introduction that the disease organism was present to a uniform degree in all the plots, it was considered likely that the differences observed in the mortalities were brought about by variations in the distribution of the pathogene in the soil. With a view to clear this point a small experiment was carried out in 1935. The seeds of the two strains were planted in the following three ways in small randomised plots repeated 4 times :—

(*a*) Two seeds of strain 468 only per hole in all the rows of a plot.
(*b*) Two seeds of strain 19 alone per hole in all the rows of a second plot.
(*c*) Two seeds made up of one seed of 468 and another of 19 in each hole in all the rows of a third plot.

It was presumed that by this treatment similar conditions of infection would be present for both the strains.

Records of their germination and subsequent weekly mortality were maintained. Since the two strains differed widely in their mode of branching and in the colour of the seed coat, it was easy to identify the type that succumbed to the disease in the mixed series. The results are given in Table II.

TABLE II.

Nature of treatment	Total No. of plants in all the repetitions	Percentage of germination					Percentage of mortality				
		Repetition				Average	Repetition				Average
		1	2	3	4		1	2	3	4	
(*a*) 468	1435	84.2	72.9	87.3	94.1	84.6	5.9	8.1	5.7	5.3	6.3
(*b*) 19	1509	89.9	88.2	83.3	94.6	89.0	42.0	64.7	42.5	44.6	48.5
(*c*) 468 only	726	80.2	83.5	84.9	93.9	85.6	8.2	11.3	6.1	9.3	8.7
19 only	841	90.2	96.9	93.7	94.6	93.9	53.0	54.0	56.0	51.0	53.5

It is evident from the above data that the germination was not affected and that even under similar conditions of exposure to the pathogene (*vide* treatment c), the strains showed themselves to be distinctly different in reaction to wilt. It would appear that by sowing the two strains in the same hole, the degree of reaction to the disease was a bit enhanced.

The strains were crossed with a view to determine their modes of inheritance of the differential response to wilt. Only one plant was

secured in F_1 which gave rise to 101 plants in F_2. Of these, 8 plants died of wilt before they reached the flowering phase, while another 17 withered late in the season after yielding a few seeds. The remaining 76 continued to be healthy till they were harvested.

It is hazardous to hypothesise from the proportion of 76 healthy to 25 wilted plants obtained in F_2 that the behaviour of these strains is influenced by a single pair of genes. For, all the plants either under 'healthy' or 'wilted' categories do not completely consist of such types. A healthy strain contains only a certain proportion of healthy plants. In the case of strain 468, for instance, only 93% will on the average remain alive and the remaining 7% will die of wilt in any case and hence will be classified under 'wilted' though belonging genotypically to the resistant group. In a similar way, all the survivors in strain 19 will phenotypically be classed under 'healthy' though of susceptible group. It was therefore essential that all the F_2 plants should be carried forward to the F_3 for assessing correctly the degree of infection.

As the proportion of mortality would be fictitiously high in very small populations, all plants with less than 20 seeds were not carried forward to F_3. 50 families from the healthy and 9 families from the wilted groups were raised in separate progeny rows. The parents were sown once after every five rows of the above series. Records of wilting were maintained for each family. When the season was over, the parents of F_3 progenies were rearranged according to their degree of mortality in F_3 populations in classes of 5% as shown in Table III.

It is clear from the nature of distribution, though small, that the reaction to wilt in the parents under study does not belong to the multiple factor type of inheritance. The higher number of readings under classes 0, 5, 10 and 15% is suggestive that dominance is at work; but the proportion betwen the number of readings above and below the 20% limit where the distribution of the two parents merge, indicates that the dominance is incomplete.

The progenies of 6 families consisting of 3 from the lower and 3 from the higher mortality classes were further studied in F_4. All the three from the former group proved homozygous for 'resistant' to wilt. But in the latter, two alone were homozygous for high mortality, the third being intermediate in the distribution (*vide* Table III). The higher proportion of homozygous cultures secured above, leads one to suspect that only one pair of factors control the differences in the reaction to wilt noticed in the parents. A study of all the F_4 generations which is under way, will finally decide this point.

TABLE III.

TABLE III.

To show distribution in the parents and their progenies.

Variety	Parental value	Percentage of mortality (mid-values)																Total
		0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	
Strain 468 (Resistant parent) ..		16	23	18	5	2													64 samples
Strain 19 (susceptible parent ..						2	12	11	11	8	5	4	4	4	2			1	64 „
F_2 (classified from F_3 behaviour)																			
From healthy plants ..		9	10	5	5	5	4	4	3	4			1						50 families
„ 'wilted' „ ..				1									1	1		2	2	2	9 „
F_4 ..	0%	15	9	2															26 sibs.
	3%	20	22	16															58 „
	9%	11	13	2															26 „
	56%		1	3	2	2	3	2											13 „
	77%					2	1	3	3	3	3	1	6	3					25 „
	77%					1		1		1		2		1					6 „

TABLE IV.

To show relationship between reaction to wilt, branching habit and seed-coat colour.

Generation	Character	Percentage mortality	No. of families studied	Total number of plants studied	Actual number of plants				Expected ratio	Value of P
					Branched		Not branched			
					Dark Brown C. S. 12	Light Brown C. S. 10	Dark Brown C. S. 12	Light Brown C. S. 10		
F_2	Wilted		1	17	9	3	4	1	9 : 3 : 3 : 1	$\wedge\wedge$ 0.9
	Living		1	70	42	13	12	3	do.	$\wedge\wedge$ 0.8
F_3	Susceptible	29, 33	2	13	All					
		22	1	31		All				
		69	1	14			All			
		24	1	75				All		
		22, 31, 82	3	86		61		25	3 : 1	$\wedge$ 0.3
		27, 72, 77, 79	4	136			97	39	3 : 1	$\wedge$ 0.3
		29, 56	2	43	28		15		3 : 1	$\wedge$ 0.3
		23, 33, 33, 37, 40, 58, 78 & 79	8	264	136	54	53	21	9 : 3 : 3 : 1	$\wedge$ 3·0
	Resistant	4, 6, 11, 14	4	54		All				
		0, 3, 4, 4	4	199		148		51	3 : 1	$\wedge$ 0.9
		10	1	20	15	5			3 : 1	1.0
		9, 19	2	76	53		23		3 : 1	0.2
		2, 7, 9	3	123	67	32	19	5	9 : 3 : 3 : 1	$\wedge\wedge$ 0.1

Susceptible Resistant.

Photograph of a field where susceptible and resistant types were grown side by side.

The existence of the difference between the parents in the type of branching and the seed-coat colour about which mention was already made, was utilised to see if any interrelationship could be detected in their progenies between these characters and reaction to wilt. It has been ascertained previously that branching and dark brown seed-coat colour differed from non-branching habit and light brown seed-coat by two pairs of genes.

The F_2 and F_3 data obtained in this cross pointed out that all the three genes segregated independently (*vide* Table IV).

The nature of resistance was also studied in the root sections of the two strains. Two main differences were noticeable between them. Firstly in the resistant plants a thick layer of suberin was produced in the periphery of the cortex. Very often the mycelium had to traverse a long distance round the stem before it could find a weak spot for the entry. In the susceptible types, on the other hand, the suberin formation was not marked. Secondly the development of the fungus, after its entry into the resistant host, was found to be very much slower than in the other type with the result only a few vessels were affected by the fungus. It would thus appear that the resistance manifested in Type 468 is consequent on the combined effect of both morphological and protoplasmic types of resistance.

The authors take this opportunity to thank the Indian Central Cotton Committe, Bombay, for permitting them to study this problem during the off-seasons of cotton crop. Our thanks are also due to Mr. K. Dharma Rajulu for preparing and examining the root sections.

PHYSIOLOGY OF THE SPIKE DISEASE OF SANDAL.

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Introduction.

From a physiological point of view, virus diseases can be classified into two groups (1) the *mosaics*, characterised by an increase in the total nitrogen and a decrease in the total carbohydrates of the foliage, and (2) the *yellows*, distinguished by a decrease in the total nitrogen and an increase in the total carbohydrates in the foliage as compared with normal plants. The spike disease of sandal, which is generally grouped under the latter, shows an increase not only in the carbohydrates as in the *yellows* but also in total nitrogen. Among other characteristic features of spike may be mentioned the exclusive occurrence of mannitol (Sreenivasaya, 1930)—which has not so far been recorded in any other virus affected plant—and succinic acid (Iyengar, 1933) in the leaves. The spike tissues also possess higher acidity (Iyengar, 1928) and are poorer in calcium (Sreenivasaya and Sastri, 1929). Nitrates which are present upto a concentration of 7 per cent. of the total nitrogen of the leaves of healthy plants, are present in much smaller quantities (about 1·8 per cent.) in the leaves of the diseased plants.

The intensive work carried out at the Indian Institute of Science during the period 1927–1933 by Sreenivasaya and his colleagues has resulted in the accumulation of a large volume of data which can be utilised for obtaining a fair idea of the nature of the metabolic disturbances consequent on the invasion of the virus principle; an attempt in this direction is presented in this paper.

Carbohydrate metabolism — accumulation of starch.

The incidence of spike brings about an abnormal accumulation of starch, the most conspicuous change occurring in the leaf. This was first noticed by Coleman (1917) and subsequently confirmed by the Institute workers. Such accumulation has also been recorded in *Dodonæa viscosa* (Sastri and Narayana, 1930), *Vinca rosea* and *Zyzyphus aenoplia*, all suffering from a malady resembling spike of sandal in its external manifestations. Besides starch, there is accumulation of the starch degradation products and of sucrose.

The earlier workers sought to explain starch accumulation by postulating a poor diastatic activity of the diseased tissues (Coleman, 1917). Later workers showed that the diastatic activity, as measured by saccharifying power, was actually very much higher (2 to 4 times) in the tissues and tissue fluids derived from the spiked plants (Sreenivasaya and Sastri, 1928). The diseased tissues also show a feeble maltase activity, and a pronounced sucrase activity, factors which favour the production of reducing sugars. An enquiry into the factors contributing towards the enhanced diastatic activity revealed, that such activity was partly due to the optimal pH conditions prevailing in the spike tissues, and partly due to the presence of activators whose specific nature was not investigated (Sastri and Sreenivasaya, 1929). The phenomenon of activation of enzymes accompanying various plant diseases is a fruitful line of enquiry. Such studies in the case of animal diseases, such as cancer, have yielded very valuable results (*cf.* Purr, 1934).

The accumulation of starch, which has been observed in the potato leaf-roll, is accompanied by phloem necrosis (Quanger, *et al.*, 1913). A similar phenomenon has not been reported in the case of spike of sandal. The cause of delayed or inhibited translocation of photosynthesised starch is, perhaps, due to the poor liquefying power of the diastase in the diseased leaves (Sastri, 1929). The enzyme from the healthy leaf tissue, on the other hand, possesses a high liquefying activity (nearly two times that of the diseased). The diseased tissues are also poor in calcium and chlorides, two constituents which help the translocation of starch (*cf.* Loew, 1903 ; Tottingham, 1919). The general dwarfing and stunting of the tissues impose mechanical barriers. The equilibrium

$$\text{Starch} \rightleftharpoons \text{products of hydrolysis}$$

which in the healthy organism leads, through the removal of the products of hydrolysis, to the depletion of starch, is disturbed ; the products of hydrolysis accumulate and as the spike tissues are poor in water content, conditions, most favourable for the accumulation of starch are obtained.

Protein Metabolism.

As physiological characteristics of the disease, may be mentioned an increase in (1) total water-soluble nitrogen, (2) basic nitrogen and (3) total amino nitrogen (Narasimhamurthy and Sreenivasaya, 1929). In the basic nitrogen fraction, a high histidine content has been recorded (Sreenivasa Rau, 1933). There is very little nitrite and ammonia in the healthy and diseased leaves ; this is in strange contrast to the higher proportion of nitrite and ammonia found in the case of spinach blight (Jodidi, 1920),

which is considered to be due to a process of denitrification. A fairly detailed investigation of the nitrogen metabolism has already been published (Narasimhamurthy and Sreenivasaya, 1924). The spike tissues also show a high proteoclastic activity. The disappearance of nitrates and their fate after the virus attack, yet remain unelucidated ; a probable course is the reduction to nitrites which immediately react with amino acids leading to the production of hydroxy acids.

Respiration and accumulation of succinic acid, hydroxy acids and mannitol.

As in the case of other virus-affected plants, the tissues of the spiked sandal also show a high oxidase and peroxidase activity (Sastri, 1929). The activity of the cell sap is enhanced (pH 4·7 — 5·0 in spike ; and 5·2–5·7 in healthy). Iyengar (1933) has shown that succinic, malic and oxalic acids are present in the diseased leaves while in the healthy tissues, succinic acid is either absent, or if it occurs at all, it is present only in traces. Further the oxalic acid and malic acid contents are lower in the healthy leaf. The ratio $\frac{\text{malic acid}}{\text{oxalic acid}}$ is nearly 0·35 in the case of healthy leaf while in the case of spike leaf, the ratio is more variable, ranging from 0·5 to 1·0. It is regrettable that figures are not available for free acids. It may, however, be assumed that in the healthy plant, the acids are more or less in a neutralised or combined form as the cell sap has a nearly neutral reaction. In the spiked tissues, on the other hand, these acids must be free, at least in part, as the cell sap is highly acid. It has been reckoned (Bennet-Clark, 1933) that at pH 5·0, 3 per cent. of the total malate is present in the acid form, while at pH 3·95, about 20 per cent. of the total malate is free acid.

To understand the mechanism of the production of these acids, it is necessary to study the respiration of the sandal leaf in the healthy and diseased conditions. Only a beginning has been made in this direction. According to the unpublished results of Keshava Iyengar (1932), the oxygen uptake of the diseased leaf, whether calculated on the surface area basis or weight basis, is only about half of that of the healthy tissue. The oxidase and peroxidase activities, on the other hand, are approximately twice as high. These observations indicate that the courses of respiration in the normal and diseased tissues are different.

In considering the respiration it is necessary to remember that in the affected tissues, there is a high concentration of sugars. It will therefore be unnecessary to consider other alternative substances, proteins or fats, which function as substrates when the tissues are carbohydrate-starved. Any scheme of respiration must also account for the production of mannitol

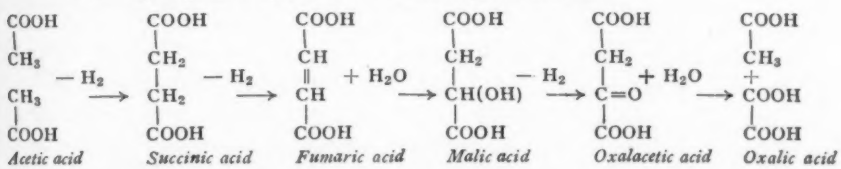
which as Sreenivasaya has shown is exclusive to spike and has not, so far, been recorded in any other virus-affected plant.

The formation of mannitol in fermenting mixtures of plant origin is known to be due to a group of bacteria. They are usually associated with souring materials (Stiles, *et al.*, 1925) such as saurkrout and silage. Mannitol is also produced from sugars by mould fungi, and Raistrick (1931) from his work on the sugar metabolism of mould fungi concluded that the first stage in the sugar breakdown is a Cannizaro reaction involving the production from two molecules of glucose, one molecule of gluconic acid and one of mannitol.

$$2\,C_6H_{12}O_6 + H_2O \rightarrow C_6H_{12}O_7 + C_6H_{14}O_6.$$

Depending on the requirements of the organisms, mannitol or gluconic acid, or both are destroyed. Raistrick made the significant observation (1931) that the quantity of mannitol produced is, to a large extent, controlled by the degree of aeration to which the fermenting media are subject. Under restricted aeration, mannitol accumulates, and the yield is considerably decreased and mannitol may even disappear under conditions of unrestricted aeration. It has already been mentioned that oxygen absorption of the spike leaves, under comparable conditions, is only half that of healthy ones. This is perhaps a factor favouring hexitol production. The products obtained by the breakdown of mannitol, gluconic acid or glucose, are qualitatively the same although slight quantitative differences exist due to their varying oxygen contents (Kay, 1920). Further decomposition of the substance might be assumed to occur according to the Neuberg scheme, as the production of 4-C acids can take place only through 2-C acids. The reaction may thus be assumed to proceed according to the following scheme :—

Mannitol → Methyl Glyoxal → Pyruvic acid → Acetaldehyde → acetic acid



Thunberg has shown that by the dehydrogenation of two molecules of acetic acid, one molecule of succinic acid is produced. Dehydrogenase activities have been shown to be present in virus-affected tissues. Thus in the case of the animal virus, bouvine pleurine pneumonia, Holmes and Pirie (1932) showed the presence of a dehydrogenase. Further reactions are self-explanatory.

The accumulation of acids is determined by the velocities of production of these acids from their respective parent substances. In the spike tissues, owing to the small concentration of calcium, the neutralisation of these acids or their removal by precipitation is hindered, thus leading to their accumulation.

The presence of de-aminase in spike tissues has recently been demonstrated by Iyengar (1935). It is assumed that the production of malic acid is due to a process of oxidative de-amination. This, however, appears untenable as numerous analyses of healthy and spiked leaves from various localities and under varying silvicultural conditions have shown that acid amides and dicarboxylic acids are present only in very small concentrations in spiked tissues and that ammonia is practically absent. The process of de-amination leading to the accumulation of hydroxy acids, appears to be of comparatively little importance. A more plausible mechanism appears to be an interaction between amino acids and nitrous acid. It has already been mentioned that in the spiked tissues the nitrates occcur only in traces. The nitrates are reduced to nitrites, which immediately react with amino acids in the acid medium leading to the production of hydroxy acids.

Conclusion.

The study of physiological characteristics of virus diseases will be found useful in (1) diagnosing the disease at an early stage and (2) in discovering resistant strains. Such studies have hardly been begun with sandal. Holmes (1932) has recently developed a technique of iodine staining which renders local lesions, due to virus, conspicuous and reveals the points of infection. Such studies may be profitably extended to sandal spike. Resistant varieties are generally characterised by low acidity and poor oxidase activities. Other factors of disease resistance, such, for instance as, phenols, glocosides, alkaloids, etc., have to be investigated. The study of active groups in tissue fluids for which a scheme was recently outlined (Sastri and Sreenivasaya, 1934) may usefully be undertaken in this connection. The possibility of nurturing sandal through appropriate hosts to impart resistance to disease has been amply demonstrated by the work of Sreenivasaya and the study of physiological characters of such plants would give results of great significance.

I wish to take this opportunity to record my grateful thanks to Mr. M. Sreenivasaya for useful discussions bearing on the text of the paper.

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BREEDING FOR RESISTANCE TO *PIRICULARIA ORYZAE* IN RICE (*O. SATIVA*).

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Introduction.

That fungus diseases cause annually a heavy loss in the important food crops is well recognised. In more advanced countries like Britain, Canada and the United States of America, there are agencies like " National Disease Surveys " which obtain and compile information regarding the range of plant diseases and crop losses due to disease. Such an organisation does not exist for India nor for any other country in the tropics. Neil E. Stevens (1933) compiling all the available information with regard to the diseases occurring in rice, mentions that over 60 diseases have been recognised and reported in available literature. In the list furnished by him, however, we find only six out of the 60 reported, as occurring in India. This does not mean that the rice crop of India is comparatively more free than in other rice growing countries like Japan, Philippines and America. This can only be due to the fact that very little has yet been done with regard to diseases occurring in rice in India. In several cases the incidence of a disease is not recognised except when it occurs in an epidemic form causing very great damage to the crop.

Piricularia in rice is known to occur in all the rice growing countries and the disease was first recognised in Japan even 200 years ago. Its existence in South India was not, however, recognised until 1918 when it appeared in an epidemic form in some portions of the Tanjore district causing over 60 to 80 per cent. loss of the crop in certain localities. The particular variety of rice, which was found attacked, *korangusamba*, had been grown for a long time in the eastern portion of the Cauvery delta in single crop lands. The trouble started only when it was taken to the western portion of the delta where people, because of its prolificacy, began to grow the variety in both single and double crop lands.

Dr. MacRae (1918) took up the study of this fungus and observed great variations in the occurrence of the disease among the different cultivated

varieties. Sundararaman (1921–1932) later continued the investigations on this disease in rice, other cereals like *ragi* (*Eleusine coracana*) and wheat and other grasses. Thomas (1930) carried out actual experiments to determine the relative resistance of several varieties of rice from the Paddy Specialist's collections to this disease. It was found that some varieties remained practically immune while others like *korangusamba* of Tanjore showed 70 to 80 per cent. infection. He also found that certain morphological characters like the size and inclination of the boot-leaf, the degree of emergence of the ear from the enclosing leaf sheath and the density of the earhead had some relationship to the incidence of the disease. His studies also showed that the disease varied in its intensity according to the environment, plots of high fertility showing greater incidence than comparatively poorer plots.

The fact that certain varieties of rice are resistant to the disease does not solve the problem as the same types cannot be grown everywhere because of the obvious differences in the requirements and conditions of rice-growing which vary from place to place, each tract having its own special set of varieties suited to its climatic and soil conditions. That this particular susceptible variety is still grown on an area of over 100,000 acres every year in Tanjore means that its yield and other agronomic qualities far outweigh the consideration of the risk due to loss caused by the disease when it does occur. Obviously the work resolved itself into one of building up by hybridisation a *korangusamba* type resistant to *piricularia*.

Scope of Work and Materials Used.—Breeders have sometimes obtained forms of crops resistant to diseases even by ordinary selections as in the case of Howard's wheats and Shaw's *rahar* (*Cajanus indicus*), but instances of resistant forms obtained by deliberate hybridisation between known parents are still rare in India. The possibilities of such work can be realised from the results recorded in this note.

Thomas's experiments had shown that two of the strains, GEB. 24 and Co. 4 evolved by the Paddy Specialist mainly for increased yields were highly resistant to *piricularia*. So these formed the parental material to be crossed with the susceptible variety, *korangusamba*. The cross between GEB. 24 and *korangusamba* was the first to be undertaken in 1926–27, and this will be dealt with in greater detail because of the conclusive and satisfactory results that have been obtained in this cross. The other cross Co. 4 × *korangusamba* was not taken up until three years later and the work has not yet advanced sufficiently.

Cross GEB. 24 × korangusamba.—86 F_1 plants were grown in 1927–28 and the descriptions of the parents and F_1s are given in Table I (Top).

TABLE I.

Cross GEB. 24 × korangusamba (1927–28).

	Average flowering duration (days)	Average height per plant Ft. in.	Average number of tillers per plant	Average emergence of earhead cms.	Average length of earhead cms.	Disposition of boot-leaf angle (degrees)	Percentage of unsetting	Remarks
T. 24 parent ..	107	3 10	54	6·0	26·8	65	9	* The unsetting here more refers to the usual spikelet sterility occurring in F_1s of certain crosses among rice varieties and not due to disease.
F_1 ..	118	4 5	73	4·1	26·8	34	70*	
Korangusamba parent ..	144	4 4	53	0·9	22·8	3	20	

Cross Co.4 × korangusamba (1930–31).

	Average flowering duration (days)	Average emergence of earhead (cms.)	Average length of earhead (cms.)
Co. 4 parent	142	4·20	24·17
F_1	136	5·33	26·05
Korangusamba parent	138	1·65	25·17

The disease was present in the F_1 though in a much milder form than in the susceptible parent *korangusamba*. Only 16 F_2 families were grown in the following year which gave a population of 30,000 plants. Elaborate notes were taken of 8,000 plants individually with regard to the degree of disease incidence by the appearance of disease spots in the leaf, the stem, and the neck of the panicle. The plants were also examined for the morphological characters like the angle of the boot-leaf, hairiness on the leaf, degree of emergence of the panicle, etc., to see if there was any correlation between the appearance of the disease and the above characters. Most of the characters except the colour of the grain exhibited complicated inheritance, the figures being not analysable into simple ratios. As an example, the inheritance of flowering duration may be mentioned here. The parents were definitely different for duration, one being markedly earlier than the other. The F_1 was more like the early parent, but in the F_2, in the several thousands of plants studied there was none which either had the same duration as the later parent or exceeded it, though a number of types were obtained definitely earlier than the early parent. The inheritance of disease infection is dealt with later, but even here the classification was made complicated because of the variation in the appearance of the disease even on the

same family grown in different fields and in addition there was greater variation in the degree of disease incidence from season to season due to climatic factors. The only line pursued was to select a large number of plants which resembled *korangusamba* in their vegetative and grain characters but which were free from the disease as identified by the absence of disease spots in the leaf, stem and neck. The F_1 and F_2 had been grown at the Paddy Breeding Station, Coimbatore, but the F_3 and subsequent generations were grown in a separate area where the Mycologist had been carrying on his experiments with this disease in the Central Farm wet lands. The assured presence of the disease from year to year in this area, its virulence alone varying according to season, removed to a great extent, the difficulty of the want of uniformity in the occurrence of the disease in the area where the crop is grown.

Every family in the F_3s and subsequent generations was grown interspaced with the susceptible variety, which served the double purpose of providing infection and as a control to estimate the degree of disease incidence in the selections. Selection and elimination of types in later generations were guided firstly by their freedom from the disease, secondly by their apparent homozygosity for other characters and lastly by their yielding capacity. The results may be summarised as below :—

1929–30. F_3.—1,200 families under observation and 450 selections made mainly for absence of the disease.

1930–31. F_4.—Most of the 450 families did not show disease but were heterozygous for other characters. Only 40 apparent homozygous families selected.

1931–32. F_5.—Disease absent in all the 40; rough comparison of yield to eliminate obviously poor ones. Only 12 retained.

1932–33. F_6. All 12 families conspicuously free from disease which was very severe in the season. One eliminated as still heterozygous for flowering duration and 11 retained.

1933–34. F_7.—Regular yield trials with *korangusamba* as the control. All facilities were provided for the families to take the infection, firstly by trampling into the transplant fields the diseased straw of the previous season, and later by spraying on the plants, just before heading, a spore suspension of the fungus.

All 11 remained free, while the control suffered badly. Six gave higher yields than the control, the increase varying from 10 to 50 per cent. as shown below :—

Strain numbers	3725	10965	10998	11340	11348	11394	Control	Percentage standard error
Yield expressed as percentages of control.								
..	134	112	154	118	116	135	100	6·64

1934–35. F_8.—Repetition of yield trials with the six, both at Coimbatore and at the Agricultural Research Station, Aduturai. Adt. 10 an improved strain from the variety *korangusamba* was used as an additional control. Season was unfavourable for the disease and the control was also practically free from the disease. Still, 4 of the strains yielded over 10 per cent. bigger yield than the control. The results obtained are given below :—

Strain numbers	3725	10965	10998	11340	11348	11394	*Korangu-samba*	Adt. 10	Percentage standard error
Yield expressed as percentages of control.									
Coimbatore	95	97	113	102	114	95	90	100	2·88
Aduturai ..	98	99	111	113	100	100	..	100	2·24

It follows from what has been stated above that some of the strains are capable of giving phenomenally higher yields of 30 to 50 per cent. than *korangusamba* when the latter suffers from the disease, and reasonably heavier yields of 10 to 14 per cent. when the latter is free and yields normally. Extensive trials have now been undertaken with these two strains at a number of centres in the districts and if the results are favourable, as they are expected to be, judging from the two years' results, we would have achieved a big advance in the production of forms by hybridisation combining yield and disease resistance.

Cross Co. 4 × *korangusamba*.—It was pointed out earlier that none of the selections from the cross GEB. 24 × *korangusamba* approached *korangusamba* in duration. The selections actually under trial are about a week earlier than *korangusamba*. The inheritance studies in other families have given indications of the existence of a positive correlation between yield and duration making it possible that a strain of the same duration as *korangusamba* would be still better in yield. It was with this idea that the cross Co. 4 × *korangusamba* was undertaken, both of them being of the same duration. Unlike in the previous cross the disease resistance was absolutely dominant in the F_1. The characters of the F_1 and the parents

are given in Table I (bottom). This cross has now come to the F_6 stage. From the examination of the material, so far made, it would appear that there are possibilities of getting results here strikingly better than from the previous cross.

Inheritance of Piricularia *Resistance.*—As regards inheritance of disease resistance, the F_2 ratios obtained in the two sets of crosses may be examined.

F_2 ratios	Diseased	Disease-free	F_1
GEB. 24 × *korangusamba* ..	248	63	Intermediate
Co. 4 × *korangusamba* ..	161	311	Resistant

From the appearance of the F_1 and the F_2 ratios in the two crosses it may be said that the nature of inheritance is different in the two crosses. Though it has not been possible to pursue the inheritance studies beyond F_3, observations on the incidence of disease would appear to show that the resistance may be a simple recessive in the first cross and a little more complicated in the other. In the former cross, selections made in F_3 for freedom from the disease straightaway began to breed pure for this character. In the latter cross, however, a certain number of disease-free selections threw diseased progenies and the obtaining of pure-breeding disease-free selections has been a little more difficult. One parent has been common for both the crosses but still the inheritance is different in the two cases. Such differences in the inheritance behaviour of a character have been recorded by Ramiah (1933) previously for other characters in rice. The difference cannot possibly be due to the organisms causing the disease in the two crosses being different physiologically inasmuch as the susceptible variety was the same in both the crosses.

Though some tangible and valuable results have been obtained from the breeding work, there still remains a considerable gap in our fundamental knowledge about this disease-producing organism. A large amount of work has been done in this fungus in Japan where the disease was observed even 200 years ago. S. Konishi (1933) and Tadayosi Nose (1935) who have examined the fungus collected from the several localities of Corea, Formosa and Japan, first cultivating them and testing their pathogenetic activity in different types, have come to the conclusion that there are different physiologic forms of the fungus. Such a work yet remains to be done in India. But the mere discovery of physiological forms need not necessarily frighten the breeder though it only makes the work of breeding resistant forms more laborious and time-taking. The experience of breeding for

resistance to diseases in other cereals has shown that several of the physiological forms fall into groups and go together so that a type resistant to one form has been found to be equally resistant to a number of other forms as well. There are reasons to believe of a similar feature in rice also.

Apart from determining the physiological forms of the fungus we want more information about the relationship between the seasonal and climatic factors and the disease incidence. Our observations on this disease during the last 8 or 9 years both here and at Aduturai definitely show that the prevailing climatic conditions, of which rainfall may be one, have some influence on the appearance of the disease each year.

TABLE II.

Relation between incidence of disease and distribution of rainfall.

Year	Coimbatore		Aduturai	
	Rainfall	Incidence of disease	Rainfall	Incidence of disease
1927—28	Below average—dry weather during early vegetative growth as well as during and after flowering.	Very mild	North-east monsoon thorough failure.	Nil
1928—29	Fairly normal during the vegetative as well as flowering phases.	Severe	Very heavy during vegetative and flowering periods.	Mild
1929—30	Normal—well distributed.	Severe	Very heavy—right through the life of the crop.	Very severe
1930—31	Above average.	Fairly severe	Heavy rains during early vegetative growth—normal later on.	Mild
1931—32	Normal and well distributed.	Mild	Heavy rains during vegetative growth and flooded conditions at the flowering period.	Nil
1932—33	Normal and well distributed.	Fairly severe	Normal and well distributed	Nil, in spite of artificial infection
1933—34	Good rains during early vegetative growth. Bright weather during flowering.	Fairly severe	Fairly good rains during vegetative as well as flowering periods.	Nil
1934—35	Much below average—dry weather during vegetative as well as flowering periods.	Nil	Much below average—dry weather during vegetative as well as flowering periods.	Severe
1935—36	Very much below average. Fairly humid conditions during the shot-blade and flowering stages. Dry weather after flowering.	Extremely severe	Good rainfall in the early stages followed by too heavy a precipitation when the crop was in flower and grains were forming.	Severe

Environmental conditions also play a part. For instance in some seasons by planting the crop early, even by a week, serious incidence is avoided. Thomas (*loc. cit.*) had found that too high fertility of the field, usually reflected in rank vegetative growth of the plant, was a predisposing cause for the occurrence of the disease. Suzuki (1933) of Japan finds that the susceptibility is in inverse proportion to the water content of the soil in which the plant grows and resistance is definitely greater in plants in flooded soils than in dry soils. Several of these points require more careful study under the conditions obtaining in South India.

Regarding resistance to the disease brought about by external characters of the host plant, Thomas's observations regarding flag-leaf, emergence of panicle, etc., since confirmed by our studies, are at best only indications that could be utilised, in making selections in cross progenies. Very little work has yet been done here with regard to resistance and cellular structure of the host plant, though Suzuki (*loc. cit.*) finds a relationship between resistance and the thickness of the outer walls and of the silicated outermost layer of the epidermal cell. He has observed also greater resistance by applying soluble silica to the soil under flooded conditions.

The knowledge about the cell sap and resistance to *piricularia* is also very meagre. In our work at Coimbatore we tried to determine the pH value of the cell sap of the resistance variety Co. 4 and the susceptible variety *korangusamba*. Certain preliminary tests showed that the sap of the resistant type was slightly more acidic but whether this has anything to do with the resistance is not known. Moreover the pH determinations have not also been done on an extensive scale.

A comprehensive attack on the whole problem of resistance should require the application of advanced knowledge of plant physiology, plant pathology and genetics but it would be safe to assume that the breeder need not actually wait until the plant physiologist or the plant pathologist has made sufficient progress to find a satisfactory explanation for all the varied phenomena connected with resistance. That plant breeders were able to produce disease-resistant forms even long before the present advance in the knowledge concerning the physiology of the organisms was reached is a sure enough evidence for this assumption.

Recently the seeds of both the susceptible variety (*korangusamba*) and the resistant variety (Co. 4) have been subjected to X-ray treatment and they have produced several mutations in morphological characters. How these will behave with regard to the physiological character of resistance or susceptibility to *piricularia* is under study.

Summary.

The disease, *piricularia*, was first noticed in Madras when it appeared in an epidemic form attacking a particular variety of rice, *korangusamba*, in portions of the Tanjore district. The studies on the fungus by the Mycologist had shown that there was a wide range of susceptibility of rice varieties to the disease, some remaining practically immune while varieties like the Tanjore one showing 70 to 80 per cent. incidence.

Work of breeding forms resistant to *piricularia* was undertaken by the Paddy Specialist in 1926. Two of the strains evolved by him, GEB. 24 and Co. 4 which were determined to be highly resistant, were crossed with the susceptible variety from Tanjore. The inheritance of disease resistance appeared to be simple in the cross GEB. 24 × *korangusamba* and more complicated in the cross Co. 4 × *korangusamba*. In the former cross disease-free selections from F_3 onwards bred pure for freedom and two of these after two years' trial both at Coimbatore and Aduturai have been found to give a big increase in yield over the susceptible variety (10–50 per cent.), the increase being more or less according as the susceptible variety gets the infection or not. Selections from the other cross are still under study and appear even more promising than the first.

We are indebted to the Superintendent, Agricultural Research Station, Aduturai, for the figures of yield trials conducted there.

Postscript.

Since the above paper was written, the yield figures of another year's trial at Coimbatore and Aduturai have become available. The season was particularly favourable for the disease, especially at Coimbatore and consequently the susceptible variety suffered badly. From the data given below, the two strains Nos. 10998 and 11340 that did well in the two previous seasons have again come out very much better than the control.

Yield figures for 1935–36.

Strain Numbers	3725	10965	10998	11340	11348	11394	Adt. 10	Percentage standard error
Yield exprersed as percentages of control.								
Coimbatore ..	136	153	180	166	165	156	100	4·00
Aduturai ..	102	96	114	118	112	108	100	4·22

DISEASE RESISTANCE IN PLANTS IN RELATION TO NUTRITION BALANCE.

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I am thankful to the organisers for having invited me to contribute a paper to the symposium on "Disease resistance in plants". Although as an Agricultural Chemist I am not directly connected with the study of plant diseases, I have had occasions to investigate certain nutritional aspects of the cotton plant in relation to its resistance to stem weevil (*Pempheres affinis*) attack and of the deterioration disease of betel vine (*Piper betle*) and to gather some data on other crops. I am, therefore, glad to have this opportunity to communicate my observations on the subject under discussion.

The expression 'disease resistance in plants' brings to mind 'disease resistance in animals'. The phenomena of animal and plant resistances in relation to nutrition may not be altogether dissimilar, but the actual methods by which immunity to insect attack and disease may be conferred or their virulence may be reduced, cannot obviously be the same or similar.

The problem of resistance in plants can be considered under two main issues which are widely different. The one is inherited and the other is acquired. The first is of primary importance and is concerned with breeding varieties which are either completely immune or possess a high degree of resistance to disease. This may be due to certain morphological or anatomical features in the plant or to the presence or absence of certain substances antagonistic to, or incompatible with, the needs and habits of the parasite. Next in importance is the maintenance of characters and capacity endowed by the breeder and inherited by the plant. It is now well known that except perhaps in their fundamentals, internal and external, wide variations in the internal economy and external manifestations are possible in plants, and that in this respect nutritional factors and climatic environment play a prominent part. It is, therefore, clear that the maintenance of disease resistance characters is closely bound up with the nature

and level of nutrition available and to the consequent physiological processes of the plant. This condition is expressible by the comprehensive but little understood term " Physiological balance". We are not yet in a position to define, with any pretension to accuracy, the term " physiological balance," much less to maintain it in the dynamic system of the plant in its equally dynamic substrate and environment. This is because our knowledge of plant nutrition is still very far from being perfect. It is, therefore, not surprising to find that conflicting results are often obtained by investigators. Nevertheless, the accumulation of data and lessons from failures and successes are necessary steps for the analysis of facts and the synthesis of knowledge.

Several instances are recorded in literature suggesting the relationship of such proximate constituents as nitrogenous compounds, carbohydrates, organic acids and several others found in plants, to their susceptibility or resistance to disease. The appearance or otherwise of these proximate constituents from the metabolic processes in plants is dependent on the nutrition available to the plant and the climatic factors that surround it. Thus the so-called " physiological balance " is the summation of the effects of climatic and nutritional factors. The climatic factor is as yet beyond man's control as a practical proposition and any discussion on it must still be in the realm of academic interest in so far as agriculture is concerned. The nutrition factor appears to be within human reach and with reasonably fair chances of control.

Considering nutritional factors in their ultimate aspect, it may be said that almost all chemical elements have been under investigation. From the accumulated data it is evident that most of the chemical elements can be grouped under two categories " essential " and " beneficial". The only difference is that some like manganese, boron, iodine are required in minute doses, while others like nitrogen, phosphorus, potash and calcium in large quantities. The former group of elements as also iron, magnesium and sulphur are ordinarily present in adequate quantities in the soils and fertilisers and irrigation waters and it is rarely that they require to be specially added. In this direction, therefore, ordinarily there is no risk of upsetting physiological balance in agricultural practice.

Physiological balance with reference to nutrition, restricts itself to the consideration of ratios between nitrogen, phosphorus, potassium and calcium which are added to the soil. Water being the vehicle, in which these nutrient elements are said to be conveyed from the substrata to the plant and as any defect either in water or in nutrient elements in the plant results in

diminished photosynthetic activity, water balance is another important factor. Since the major elements of nutrition are capable of forming acids and bases, the *balance* in respect to these becomes in effect *acid-base-balance.* Thus the discussion on physiological balance or nutritional balance in relation to the susceptibility or resistance of plants to disease may be narrowed down into two main issues, namely, *acid-base-balance* of ash and water balance. The influence of these two factors on disease resistance manifests itself in several ways and can be measured by several standards. For purposes of this discussion, the yield of crop is taken as the standard of measurement.

I. The Resistance of Cotton Plant to Damage by Stem Weevil (Pempheres offinis) :

(*with Y. D. Wad and M. Suryanarayana*).

In this section the salient features of investigations over eight years on the factors concerned in the resistance of cotton plant to stem weevil damage are reported.

The problem is briefly this : The stem weevil lays its eggs on the hypocotyl regions of the cotton plant and covers them with a gummy secretion from its body. The egg hatches and the larva burrows into the stem. The insect feeds on the sap and soft portions of the plant, and makes in the stem irregular tunnels of all shapes and sizes. Eventually, the grub settles down to pupate and finally emerges by cutting through the bark. Although the insect exhibits a definite preference to the cotton plant as a host, it does not seem to be attracted or repulsed by individual plants in a cotton crop. So far as is known, the selective faculty of the insect is limited to the softer portions of the stem and possibly to such plants as may reasonably be expected to provide suitable nourishment to the young one. The development of the grub in the plant, and the repair of the damage caused to the plant tissues, cause a heavy drain on the plant sap. Plants that are unable to meet this drain succumb early in their career. Those that are more favourably situated strengthen themselves by developing galls round the seat of injury. Sometimes as many as half a dozen or more galls are seen on a single plant. The escape from destruction due to mechanical injury depends on the balance left over by the developing grub.

The attention of the writer was first drawn to the problem in 1923 by the Government Entomologist at Coimbatore. In a cotton crop belonging to the mills at Virudupatti nearly fifty per cent. of the plants succumbed to stem weevil attack, while in the neighbouring ryot's field there was practically no damage. The variety of cotton in both the fields was the

same (Karunganni—*G. indicum*). At that time the phenomenon of attack in one field and absence in another was strongly suggestive as being due to specific substance or substances in the cotton plant which offered preferential attraction to the insect. Subsequent investigations by the Entomologist and the Cotton Specialist, Coimbatore, and my own observations have shown so far that there is nothing of the kind.

Chemical analyses of a large number of plants made in 1923 did not indicate appreciable differences in the absolute amounts of the several organic constituents of healthy and attacked plants. There were, however, significant differences in the ratio $K_2O : P_2O_5$.

$K_2O : P_2O_5$	for stem weeviled plants in manured field ..	4·96
Do.	for plants in ryot's field	9·64

This clue was followed up in the next season by carrying out analyses of individual plants from field No. 13, Central Farm, Coimbatore. The potash : phosphoric anhydride ratios were as below :

	Field No. 13.	$K_2O : P_2O_5$
1.	Plants attacked by stem weevil, but living with galls developed	5·56
2.	Unattacked healthy plants	4·95
3.	Stem weevil attacked and dead*	4·50

The ratio for the plants that had the attack, but were living, is significantly higher than that for the plants that were attacked and dead.

This confirmation of the preliminary observation prompted further study. This was done in a subsequent season by submitting to individual analyses a large number of Cambodia (440) plants kindly supplied by Mr. V. Ramanathan, the Cotton Specialist, Coimbatore. The investigation was also extended to the study of the different parts of healthy and attacked cotton plants. Here again no appreciable differences were noticed in the acidic or basic constituents of the ash of healthy and attacked plants except in the case of magnesia which was consistently higher in healthy plants. The significance of this is not clear. The ratios were similar to those found previously. These are given below as excess or deficit for healthy over attacked plants.

	K_2O/P_2O_5	$K_2O + CaO + MgO/P_2O_5$
Healthy *minus* attacked : Leaf ..	+ 0·30	+ 1·69
Healthy *minus* attacked : Stem ..	− 0·62	− 3·14
Healthy *minus* attacked : Root ..	+ 0·45	+ 0·61

Assuming that normal absorption and assimilation obtains in the healthy plant, it will be noticed that in the attacked plants, there is a deficit of basic constituents in root and leaf and an excess in the stem. The pH values of the leaf and stem extracts do not, however, indicate this difference in a pronounced manner as will be seen from the figures below :

	pH Values	
	Healthy	Attacked
Stem	6·11	6·18
Leaf	5·37	5·41

If, as a result of stem weevil attack, there should be difference in the absorption and assimilation of mineral constituents by the cotton plant and if the struggle by the plant to repair damage is expressed by the nodular swelling, differences in the ratios for the stem and its gall should be visible. That it is so is seen from the figures below :

	K_2O/P_2O_5	$K_2O + CaO + MgO/P_2O_5$
Healthy stem ..	3·29	9·02
Attacked stem ..	3·91	9·62
Gall on attacked stem ..	3·58	6·48

These results suggested that by controlling ratios of the major nutrients applied to the soil, differential absorption might possibly be induced and that further indications might be secured if such plants were exposed to stem weevil attack. To this end, over a hundred large size flower pots were filled with soil and, after applying superphosphate and potassium sulphate to give different $K_2O : P_2O_5$ ratios, the pots were sown to cotton. When the seedlings were about a month old, the pots were distributed at random in a large field of cotton crop, the object being to freely expose all plants to stem weevil attack. At the end of the season counts were made with the following results :

Treatment	Percentage of plants		
	Healthy	Attacked and recovered	Attacked and dead
Soil alone ..	42	29	29
Soil + farmyard manure ..	35	41	24
Soil + $K_2O : P_2O_5$: : 1 : 1 ..	10	76	14
Soil + $K_2O : P_2O_5$: : 1 : 4 ..	12	65	23

The results of two sets of experiments on the field scale are interesting. One set of field tests was carried out in the old permanent manurial plots in which the plots received the same differential fertiliser treatment continuously for twenty years. In the second set the new permanent manurial plots were under differential feritliser treatments for only three years. The first set, therefore, approximates closely to the rigours of solution cultures without their attendant disadvantages. The second set approximates closely to fertiliser treatment under normal field conditions.

The results are given in the table below :

Treatment	Series of plots I Old Permanent Manurials				Series of plots II New Permanent Manurials			
	Attacks %	Deaths %	Exchangeable bases		Attacks %	Deaths %	Exchangeable bases	
			Total	Potassium			Total	Potassium
Unmanured ..	87·0	7·3	23·3	0·3	89·0	3·6	28·6	0·8
N + K + P ..	84·5	3·8	24·2	0·4	92·1	3·5	30·4	1·0
K + P ..	85·5	4·0	23·0	0·5	83·1	2·8	29·4	0·8
P ..	84·0	4·1	22·6	0·3	84·6	2·4	32·2	0·8
K ..	80·0	1·8	23·4	0·6	81·7	2·5	32·3	1·0
Cattle manure ..	90·3	2·1	20·0	0·4	81·8	0·9	31·7	1·2

The percentage attacks and deaths are as observed by the Government

Entomologist, Coimbatore. It will be seen that plants under all treatments were attacked by the stem weevil. This lends support to the view previously expressed, *viz.*, that the stem weevil visits all plants indiscriminately. Deaths are proportionately very small and the differences are too small to justify serious consideration. It was unfortunate that the experiments could not be repeated in another year. However, the indications are generally in agreement with the previous discussion on balance and ratios. Particularly is this more in evidence in series II than in series I. In the latter series the differential treatments continued over a long series of years and the effects are, therefore, of an emphasised nature and consequently in this set of plots the balance was, perhaps, in an unstable equilibrium. An interesting observation is the effect of cattle manure in series II. The attacks and deaths were low and the values for exchangeable bases were as good, if not better than, when artificial fertilisers were applied. For a field experiment in the tropics and a crop like cotton whose water balance is delicately poised, the agreement between laboratory results and field behaviour appears to be good. The results with cattle manure as a factor in maintaining nutritional and water balance are suggestive.

The analyses of another crop in series I plots show a general relationship between manurial treatment and leaf composition. The deficiency in N, P and K in the soil is reflected by a decreased content of that element in the leaf, but a constancy in the ratios of nutrients was evident throughout the growth period of the plant. The magnitude of the ratios, however, varied with the nature of the fertiliser treatment given to the soil.

II. Betel Vine Investigation:

(*with T. Lakshman Rao and M. R. Balakrishnan*).

The problem in regard to betel vine was this. In the betel vine areas of the Noyel valley in the Coimbatore district there had been a gradual and general deterioration in the growth and yield of vines. The production of the leaves was reduced, their size became smaller and the side branches became fewer and lankier. The area was situated in a low level and the soil was in a state of supersaturation with water for about eight months in the year. The conditions of betel vine cultivation are such, that high humidity in the interior of the crop was a feature. Root Knot eel worm—*Caconema* (*Heterodera*) *radicicola* and among several fungi, a pathogenic fungus *Rhizoctonia*, which caused blackening of the stem at about a foot from the ground level and then death, were noted. At first it almost looked that the eel worm and the fungus were responsible for the damage. In a co-operative investigation for five years by the Chemist, Entomologist and Mycologist, it was conclusively proved that the diseased condition of

the betel vine crop was due mainly to bad soil conditions and that all external manifestations of disease attributed to earth worms, presence of pathogenic fungus were but an index of unhealthy soil conditions. The betterment in soil conditions improved the yield to nearly normal.

The point of interest for purposes of the present discussion lies in the effect on water balance in the plant. The water balance in the soil was reflected in that of the plant and also in its mineral composition as can be seen from the following analyses of healthy and diseased plants :

Composition of ash of leaves.

	Composite sample from all plots		Sample from plots	
	Healthy	Diseased	Best situated	Worst situated
	%	%	%	%
$Fe_2O_3 + Al_2O_3$ ..	0·66	0·44	0·95	0·68
CaO ..	1·82	2·02	0·95	0·55
MgO ..	1·32	1·79	1·28	0·74
K_2O ..	5·13	3·83	5·83	2·70
P_2O_5 ..	0·66	0·55	0·65	0·42

The water content of leaves as determined by drying them at 100° C. and their nitrogen contents were higher for leaves from healthy plants than from diseased plants.

Sample	% Water content in leaf		% Nitrogen content in leaf	
	Healthy	Diseased	Healthy	Diseased
From all plots in September ..	84·04	76·64	3·04	1·75
Plot 3, October ..	79·26	76·60	2·64	2·32
Plot 4, October ..	83·52	76·21	2·74	2·40
Plot 3, November ..	81·33	79·17	2·53	2·38
Plot 4, November ..	85·60	72·50	2·78	2·63

It would appear that the state of soil-water relationship affected the water balance in the plant. It is difficult to say whether the absolute water content in the diseased leaves was definitely low or whether the water existed in a "*bound*" state and could not be removed at 100° C. The lower nitrogen content of the diseased leaves does not suggest a relatively higher content of colloid material which would be expected to imbibe and "bind" water. The existence of hydrophilous carbohydrates might be equally responsible. In any case, the interesting and instructive point that has emerged from the available data is that the effect on the plant of supersaturated condition of the substrate in regard to water, is not merely defective root aeration, but to induce defective water balance in the leaf to the detriment of the general health of the plant.

III. Abnormal Leaf Fall of Rubber Trees.

In this Section are recorded the results of periodical analyses of rubber tree leaves relating to normal and abnormal leaf-fall. The data reported do not belong to any specific investigation by the author, but refer to the analytical work done, as Agricultural Chemist, Coimbatore, on samples sent in by Mr. D. G. Munro, the then Deputy Director of Agriculture, for planting districts. The data are presented here on account of their interest in respect to the potash content of the leaves.

Percentage of potash on ash of leaves for six trees in one season.

Particulars	Normal leaf-fall leaves	Abnormal leaf-fall leaves
Tree 1	10·61	20·70
Tree 2	5·32	21·29
Tree 3	8·58	21·03
Tree 4	11·26	26·76
Tree 5	13·61	23·15
Tree 6	14·40	25·17

Periodical collection during season 1924–25.
(Mooply Experiment Station. Average for 6 trees.)

Normal fall leaves		Abnormal fall leaves	
% K_2O on		% K_2O on	
Leaf	Ash	Leaf	Ash
0·9	12·1	1·7	25·4
1·1	12·6	1·7	19·0
0·9	10·8	1·5	25·2
		1·5	22·9
		1·6	19·7

The leaves were from the same trees. Those relating to abnormal leaf-fall had an infestation of phytophthora fungus. The association of abnormal leaf-fall and fungus with a consistently higher concentration in the leaf is the opposite of what has been seen in the case of betel leaf. This is suggestive that potash should not always be considered as an antidote for disease.

IV. General Discussion.

It is evident from the foregoing that, genetic constitution apart, the susceptibility or resistance to disease and insect attack is governed by the internal condition of the host plant and that nutritional and water balance play a prominent part. Lees[1] has cited several instances to show the varying susceptibility of plants to disease and insect attack, due to the influence of soil conditions and growth of the plant. McRae and Shaw[2] at Pusa have shown that the incidence of wilt (*Fusarium vasinfectum*) of *Cajanus* with the type of manurial treatment. According to them, manuring with superphosphate or cattle manure increased wilt. A green manure like sann hemp reduced wilt affection, while the addition of phosphate with the green manure increased it. The effect of phosphate might be the disturbance of acid-base ratio. Wallace[3] concludes that leaf scorch in apple trees arises generally from defective nutrition, but that in many cases it develops

as the result of unsatisfactory conditions of water-supply within the plant. Grubb[4] discusses the effect of potash fertilisers upon apple trees as being able to prevent the development of leaf scorch.

The study of the influence of manures and fertilisers on acid-base balance in plant ash and on water balance within the plant may not only explain how substances like potash salts prevent or moderate damage from disease, but would also enlarge our knowledge on this aspect of disease resistance in plants. Fertiliser salts exercise only a very limited action on the pH of the plant juice but considerably influences its buffer capacity, and also affect the acid-base balance of plant ash. It would appear that fertiliser salts can also influence the state of water balance in the plant by altering the proportion of bound water and the hydration or dehydration capacity of the tissues.

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SOME NOTES OF THE INCIDENCE OF DISEASE AND RESISTANCE TO PATHOLOGICAL AND OTHER ADVERSE CONDITIONS IN CROPS OF SIND.

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Introduction.

The possibility of controlling insect pests and attacks by parasitic fungi by means of host resistance has recently come within the purview of the plant breeder and although such studies are only in their infancy they indicate great hope for advances in this direction. In order to plan a systematic attack on this problem, it is essential to obtain information and collect data on the various forms existing in crop plants, isolation of unit species in the more promising varieties or types, study of hereditary characters and the reaction of the plant to diseases, pests and environmental conditions. These data have been collected during the course of plant breeding work on the more important crops of Sind and whenever possible observations have been recorded on the incidence of disease and attack by insect pests and mortality due to other causes, *e.g.*, adverse soil and weather conditions. As a result of these observations, it has been possible to eliminate certain types or varieties which were found to be susceptible to these troubles and at the same time discard certain progeny lines which showed greater susceptibility than others. In the plant breeding work at the Agricultural Research Station, Sakrand, a few plants are selected in all the strains every year which conform to the type and are free from disease. The selfed seed of the selected plants is grown in line cultures in the following year. The best line culture is retained for further testing next year. This continuous maintenance of single plant tradition both in the selected strains and hybrids, has greatly contributed to the production of improved strains suited to the local conditions.

Varietal adaptation and stand of crop in cotton.—In Sind it has been found that the germination of cotton is as a rule good but it is difficult to maintain a good stand in exotic cottons. Considerable mortality takes place for one or more causes among which may be mentioned high temperatures, hot winds, white-ants, root rot, etc. Counts of final stand showed

that 25% of the plants survived in Egyptian cottons, 58% in Punjab Americans and 75% in the improved Sind deshi 27 W. N. In botanical selection for crop improvement, hardiness to withstand local conditions is an important characteristic to be taken into consideration. A good stand after the crop is well established is an index of hardiness and ability to withstand adverse soil and weather conditions and also insect attack especially by white-ants and fungoid diseases, like root rot. In a varietal test conducted at the Seed Farm, Mirpurkhas, in 1932–33 the number of surviving plants was counted at the time of the first picking. The following table shows the results :—

Due to	Stand of crop. Mean No. of surviving plants				General Mean	Standard error of mean	Significance by 'Z' test	Critical difference for significance. (No. of plants per acre)
	27 W. N.	289 F-1	285 F-2	285 F-21				
Per plot ..	975	579·5	765·1	551·5	717·7	219·25	Significant by 'Z' test on 5%	1793·2
Per acre ..	15600	9272·0	12242	8824	11484·5			
Percentage on general mean ..	135·8	80·7	106·6	76·9	100			
Percentage on control ..	100	59	78	56	..			

It will be seen that the improved Sind deshi strain 27 W. N. in point of stand of crop ranks the highest among the varieties under test followed by the improved Sind American strain 285 F–2. This experiment was repeated in the following year 1933–34 and the following results were obtained :—

Due to	Stand of crop. Mean No. of surviving plants				General mean	Standard error of mean	Significance by 'Z' test	Critical difference for significance. (No. of plants per acre)
	27 W. N.	4 F-98	289 F-1	Acala 27				
Per plot ..	1004·5	693·5	800	625·1	780·7	451·6	Significant on 5% level	4518·56
Per acre ..	21475	14834	17112	13371	16698			
Percentage on general mean ..	128·6	88·8	102·4	80·0	100			
Percentage on control ..	100	69·0	79·6	62·2				

It will be seen that in this season also the improved Sind deshi ranked first in point of stand of crop ; but the stand of the crop was on the whole very much greater in 1933–34 than in 1932–33. The following table will show the relative stand and the yield of the varieties under test in the experiments described above.

1932—33				1933—34			
Variety	Relative stand %	Yield of seed cotton		Variety	Relative stand %	Yield of seed cotton	
		Per acre in lbs.	Per plant in grams			Per acre in lbs.	Per plant in grams
27 W. N.	100	1620·0	50·0	27 W. N.	100	1571·2	35·4
289 F—1	59	1321·6	64·8	289 F—1	80	1020·0	28·9
285 F—2	78	1121·6	41·6	4 F—98	69	1055·0	34·2
285 F—21	56	840·0	43·2	Acala—27	61	508·0	18·4

It will be seen that the improved Sind deshi, having the highest stand, gives the most yield and although the stand of crop was greater in 1933–34 than in the preceding season, the yield, on the whole, was less than that of previous year. This experiment was repeated in the third season 1934–35. The results are briefly summarised in the following table.

Due to	Stand of crop. Mean No. of surviving plants				General mean	Standard error of mean	Significance by 'Z' test	Critical difference per acre for significance. (No. of plants per acre)
	27 W.N.	289 F-1	285 F-2	4 F-98				
Per plot ..	402·6	340·1	365·3	408·3	379	253·1	Not significant	
Per acre ..	25763·5	21656·0	23381·3	26133·3	24233·5			5237·1
Percentage on general mean ..	103·1	89·3	96·4	107·8	100			
Percentage on control ..	100	84·0	90·7	101·4	..			

It will again be observed that although the stand of the crop obtained during 1934–35 was greater than that of the preceding two seasons, the yield on the whole was much lower especially in the case of 27 W. N. and 4 F–98 as shown hereunder :—

Variety	Relative stand %	Yield of seed cotton	
		Per acre in lbs.	Per plant in grams
27 W. N.	100·0	628	11·03
289 F—1	84·0	676	14·30
285 F—2	90·7	731	14·20
4 F—98	101·4	621	10·76

The low yield in spite of good stand of crop was probably due to heavy shedding of buds and flowers caused by the continuous cloudy weather in the months of July-August and part of September. The cottons which were either very early in flower, *viz.*, 285 F–2 or very late in flowering, *viz.*, 289 F–1 were less affected than the other two cottons, *viz.*, 27 W. N. and 4 F–98 which occupy an intermediate position in point of flowering period. This is a case in which seasonal variations have brought about reduced yield in spite of good stand. It may be noted that in order to cultivate exotic cottons in Sind successfully, it is essential to obtain a good stand.

The other important characteristic which is most desirable in a cotton variety to be grown in Sind, is its resistance to a peculiar condition of the leaf caused by jassids, or other organisms or otherwise commonly known as the 'red leaf' disease. Most of the imported American cottons possessing leathery and glabrous leaves have been found to be so affected. The Punjab American cottons are also affected but some of the strains derived from them and selected for resistance to this condition have proved to be very suitable for growing in the Barrage areas of Sind. Thus the selected strains of Sind American cotton, *viz.*, 289 F–1 and 4 F–98 are the best strains in this respect so far obtained. These selected strains are being multiplied.

Another peculiar condition which has been noticed among the Egyptian and Sea Island cottons tried in Sind is the 'leaf crinkle'. Most of the varieties so far tried in Sind, *e.g.*, Sakel, Pima, Yuma, Maarad, and Sudan cottons, have been found to be so affected. The only varieties which were found to be less affected are :—(1) Ashmoni, (2) Zagora Melaky, (3) Sea Island. In these three varieties selection has been made for resistance to this condition and as a result of the work of selection and acclimatisation three improved strains of cottons, *viz.*, Ashmoni 37, Boss III–16 and Sea Island 2–4 have been produced, which can be grown successfully in Lower Sind.

Incidence of long smut (Tolyposporium filiferum) *of jowar* :—This fungoid disease is common in Sind and the extent of damage varies with the season. Counts taken in cultivator's field on 500 earheads in the year 1926–27 showed that the smut-affected grains in an earhead varied from 0 to 70 grains, the mean being 8 grains. Considering the large number of grains formed in an earhead exceeding 2,000 in the local unselected variety, the percentage of smut attack is indeed small. In the same year the counts of smutted grains were taken in line cultures of 14 Sind varieties and results are shown hereunder :—

Variety	No. of grains per earhead		Smutt %	Variety	No. of grains per earhead		Smutt %
	Sound	Smutted			Sound	Smutted	
Allakh ..	3575	13·2	0·37	*Bagdar* ..	3767	70·2	1·83
Sakrand *Local* ..	3677	15·1	0·41	Janapur Red ..	2908	66·4	2·23
Acho Bazigar .	1913	10·4	0·54	*Acho Kartuho*	2854	93·2	3·16
Achi Turi ..	3122	16·8	0·53	*Saoro Kartuho*	2145	35·9	3·40
Gulmahomed Garhi ..	2149	19·8	0·91	Garho Bazigar	2511	92·5	3·51
Acho Kodri ..	2284	21·9	0·95	Garhi Turi ..	1438	68·2	4·52
Navabshah *Local* ..	2623	25·8	0·71	Red Sathri ..	2477	169·0	6·42

It will be seen that some varieties were more affected by 'long smut' than others and the highest attack by 'long smut' was 6½%. Breeding of resistant strains in the more high yielding varieties shown in italics in the above table has been taken in hand.

In 1932–33 there was a severe attack of 'long smut' in grain jowars which were in flower towards the end of August or early in September. Two varieties, *viz.*, Bagdar No. 1 and Saoro Kartuho No. 1 which, as a rule, mature late were badly affected in plots which had been sown in the last week of June (30th June) but were comparatively free from the disease in plots sown in the second week of June (7th to 12th June) a fortnight earlier. All the *jowar* varieties which were in flower before 3rd week of August seemed to have escaped this trouble. Similar observations were recorded in the following year. In a plot sown on 8th June with Bagdar variety,

28% of the earheads were affected with long smut while in another plot sown on 22nd June a fortnight later than the former with the same variety, 44% of the earheads were so affected. These observations indicate that the most suitable date for sowing late maturing varieties, *viz.*, Bagdar, Saoro Kartuho and Acho Kodri is during the first ten days of June so as to avoid the attack of long smut.

Incidence of stem borer (Chylo simplex) *of jowar.*—As a rule jowar crop is practically free from borer attack but this pest causes damage in certain seasons. During the last 9 seasons, the crop was badly affected in two seasons only, *viz.*, 1930–31 and 1933–34 and yield was greatly reduced. Counts of affected plants were taken in 1933–34 on a number of established strains and hybrids and most of them showed 47 to 71% attack. Only two strains, *viz.*, Allakh No. 2 and a hybrid derived from it, *viz.*, Allakh × Torh No. 5–54–3–39–109 showed 25 and 29% attack respectively. The former is a good quality jowar and the latter has both high yield and superior grain. The value of a variety cannot be determined entirely by its ability to resist attacks of pests or parasitic fungi but other physiological characters such as yield, quality of grain, etc., demand attention.

Study of 'rati' (brown spot) and semi-sterility in rice varieties.—The brown spot disease of rice known locally as 'rati' caused by *Helminthoporium oryzæ* has been a very serious disease in Upper Sind for a long time. It affects the crop in the later stages of its growth in September and causes sterility of the earhead and shrivelling of the grain thus reducing the ultimate yield of the crop. The disease is extensively carried from season to season by means of seed, infected with the fungus spores, but secondary infection from the soil does take place. The attack of the disease was found to be most severe (*a*) on late varieties of rice, (*b*) in low lying and shady areas, (*c*) under humid conditions with low temperatures. To check the spread of the disease, several methods of seed treatment were tried. They did check the primary infection, but not the secondary infection from the soil, air and irrigation water. Breeding of 'rati' resistant varieties has given quite promising results as shown in Tables I and II. Sterility, commonly known in Sind as 'khas', is a condition in which the grain fails to set; wholly sterile heads are rare in Sind even in a year when 'khas' is prevalent. The percentage of sterile flowers varies in different varieties and from season to season. In spite of this variation this character has been found to be hereditary and for convenience varieties have been grouped as follows :—

(1) Varieties with low sterility—Produce less than 10% sterile grains.
(2) Varieties with medium sterility—Produce between 10–20% sterile grains.
(3) Varieties with high sterility—Produce more than 20% sterile grains.

TABLE I.—1933–34.

Variety	Rati attack %		Sterility %		Yield of main culm earhead in grams		No. of earheads examined in crosses
	Cross	Check Kangni-27 Parent (Mean of 100 earheads)	Cross	Check Kangni-27 Parent (Mean of 100 earheads)	Cross	Check Kangni-27 Parent (Mean of 100 earheads)	
			Plot No. I.				
Kangni 27 × Kolumba cross.	%	%	%	%			
28—889—56	11	21	9	16	3·22	2·21	400
38—845—70	13	19	10	15	3·47	2·45	1800
38—845—16	15	13	11	24	3·55	2·45	900
			Plot No. II.				
	%	%	%	%			
38—845—86	25·5	30	9	12	2·90	2·01	200
34—267—51	26	38	24	12	2·34	1·45	1100
Kangni 27 × Torh No. 1 cross.							
16—1—28	34	26	12	13	1·76	1·81	100

TABLE II.—1934–35.

Variety	Rati attack %		Sterility %		Yield of main culm earhead in grams		No. of earheads examined in crosses
	Cross	Check Kangni 27 Parent (Mean of 100 earheads)	Cross	Check Kangni 27 Parent (Mean of 100 earheads)	Cross	Check Kangni 27 Parent (Mean of 100 earheads)	
Kangni 27 × Kolumba cross.	%	%	%	%			
28—889—56	5	8·5	12	14	3·29	2·85	200
38—845—70	5·3	7·5	8·7	14	4·40	2·67	300
38—845—16	5	7·5	8·0	12·5	4·44	2·76	500
34—267—51	7	8	8	12	3·78	2·86	200
Kangni 27 × Torh No. 1 cross.							
16—1—28	6	7	11	10	2·34	2·79	100

Observations were recorded by the Rice Mycologist on incidence of 'rati' and 'khas' in some of the line cultures grown in the Botanical Section area in the year 1933–34 and 1934–35. Main culm earheads were examined in some established rice strains and hybrids.

Taking into consideration the characters of the parents, it may be noted that Kolumba 184 is more susceptible to semi-sterility and 'rati' disease than Kangni 27 which in point of yield also is superior to Kolumba 184 and Torh. Hence in studying the incidence of attack, Kangni 27 which was grown side by side has been kept as a check. It will be seen that the hybrid strains show on the whole less attack by 'rati', less sterility per cent. in the earhead and higher yield. By proper selection of plants, it has been possible this year to show a distinct improvement in the more promising types as regards resistance to 'rati' over the results of last year. Breeding of resistant strains is being done by the Rice Mycologist in collaboration with the Botanical Section. Testing of resistant progenies for several generations in the field and artificial inoculation to test susceptibility will form the main lines of work.

Tolerance of rice varieties to 'kalar' in the soil.—Preliminary observations on tolerance of rice varieties to presence of salt in the soil were made in the year 1932–33. Three varieties of rice, *viz.*, Torh, Lari and Kalangho well known for their 'kalar' resisting power were tested with the improved strain Kangni 27 in a plot of land badly infested with 'kalar' and measuring $\frac{1}{8}$th of an acre in four replications, the unit plot being 1/128th of an acre. The following yields were obtained :—

Due to	Mean yield in lbs.				General mean	Standard error	Whether significant by 'Z' test	Critical difference by Z test. Yield in lbs. per acre
	Kangni 27	Torh	Lari	Kalangho				
Per plot ..	9·88	9·69	10·25	8·06	9·47	6·22	Not significant	
Per acre ..	1254·4	1228·8	1305·6	1100·8	1211·9			635·4
Percentage on general mean	103·5	101·4	107·7	90·8	100			

The following stand of crop as judged by the number of plants surviving, was obtained :—

Variety	Stand per cent. surviving plants	Variety	Stand per cent. surviving plants
	%		%
Kalangho ..	76·6	Kangni ..	59·0
Lari ..	73·9	Torh ..	68·7

In the following year 1933–34 five main varieties of rice, *viz.*, Kangni 27, Prong 36, Bengalo, Torh and Jajai 77, along with seven selected strains of rice hybrids, were grown in strips of 7 rows each in a plot of land measuring 2/5th of an acre badly infested with 'kalar'. The following results were obtained :—

Variety	stand %	Yield of grain per strip in lbs.	Variety	Stand %	Yield of grain per strip in lbs.
			Kangni × *Torh cross.*		
				%	
Torh ..	57	36·0	16–1–28–11	57·6	36·0
Bengalo ..	55	31·0	16–1–28–80	54·7	33·4
Kangni 27 ..	45·8	32·4	*Kangni* × *Kolumba cross.*		
				%	
Jajai 77 ..	30·2	18·2	38–845–70	43·6	27·4
Prong 36 ..	25·3	19·4	38–845–86	36·5	26·4
			38–845–16	34·1	26·4
			28–889–56	25·8	14·4
			34–267–51	26·2	19·0

As these results pertain to single plots it is difficult to judge accurately the 'kalar' resisting power of the varieties, but these preliminary observations do indicate that high quality rices, namely, Jajai 77, Prong 36 and hybrid 28–889–56 with the exception of Bengalo show least resistance to 'kalar'. Torh variety and hybrid strains derived from it show the highest stand per cent. and greater yield of grain.

During the year 1934–35 five main varieties of rice, *viz.*, Kangni 27, Jajai 77, Prong 36, Bengalo and Torh together with five selected strains of rice hybrids were grown in duplicate strips of ten rows each in a plot, measuring 2/5 acre, badly infested with 'kalar'. The following results were obtained :—

Average of two plots			Average of two plots		
Variety	Stand %	Yield of grain per strip in lbs.	Variety	Stand %	Yield of grain per strip in lbs.
			Kangni × *Kolumba cross.*		
Kangni 27 ..	80·5	42·0			
Jajai 77 ..	83·0	43·0	38—845—70	66·5	22·5
Prong 36 ..	82·5	40·5	38—845—16	62·0	37·0
Bengalo ..	80·5	40·5	28—889—56	58·0	37·0
Torh ..	72·0	43·5	34—267—51	59·0	27·0
			Torh × *Kangni cross.*		
			16—1—28	68·0	43·0

It will be seen that the Torh variety and the cross Kangni × Torh, in spite of low stand, have given the highest yield. In the preceding year these two rice varieties had stood highest in point of both stand and yield. The other rice types which stand 'kalar' well are Kangni and Bengalo confirming the observations made last year.

Susceptibility to frost.—Rabi (cold weather) crops are liable to the attack of frost in Sind in certain seasons. It has been noticed that frost occurs in two out of five seasons. There are two periods when frost may occur, (1) end of December—early January, (2) end of January—early February. The latter period is more frequent and does the most damage. Wheat is the major *rabi* crop in Sind. All varieties of wheat so far tried in Sind are affected by frost if the plant is in flowering stage at the time of the occurrence of frost. Hence, in order to escape frost in Middle and North Sind indigenous wheats should be sown in the latter part of November so that they come in flower when the danger of frost has passed. Wheat crop is sometimes affected by rust disease, especially the late sown crop. Several varieties of wheat have been tested. The improved strains of wheat selected from indigenous crop have been found to be less susceptible to rust than the local selected varieties. Several varieties of wheat from different parts of India have been tested. Pusa 114 wheat has been found to resist rust attack considerably and is decidedly superior to other improved strains evolved in Sind and the Punjab. Owing to the low prices obtained for wheat during the last few years attention has been

given to the investigation of alternative money crops to wheat. Cruciferous oil-seeds have been found to be much more profitable than wheat, provided they are not hit by frost. Sowing date experiments have been conducted at Sakrand for North and Middle Sind and the results indicate that early varieties of Torio (*Brassica Napus* var. *dichotoma*), *viz.*, Lyallpur types and Kazi Ahmed variety should be sown in September so that they are actually harvested before the frost occurs. On the other hand, late types of Torio, *viz.*, Halla and Mirpurkhas are best sown in end of October so that they will come in flower after the frost period has passed. Similarly, rape-seed (*Brassica campestris* var. *Sarson*) should be sown during the first fortnight of November since earlier sowings are as a rule affected by frost and mustard (*Brassica juncea*) is best sown in the month of December. Late flowering varieties of linseed, *viz.*, Pusa H-68, Pusa H-12 and Halla escape frost when sown in the end of October. The investigations carried out at the Agricultural Research Station, Sakrand, clearly indicate that oil-seed crops possess a wide range of sowing period which enables the cultivators of these crops to make full and economic use of irrigation water and at the same time arrange the time table of sowings in such a way so as to avoid frost attack and get the best returns.

Frost resistant types of oil-seeds.—No frost resistant types were found in Torio (*Brassica Napus* var. *dichotoma*), Jambho (*Eruca sativa*), Rape (*Brassica campestris* var. *Sarson*) and Mustard (*Brassica juncea*) varieties imported from different parts of Sind and other Provinces. The safflower crop is affected by frost in Sind even before the flowering stage. Among the various types of safflower obtained from Pusa, Deccan and Gujrat Pusa types Nos. 9, 17, 18 and 19, and Dholka No. 1 and 2 were found to resist frost attack.

STUDIES IN DISEASE RESISTANCE IN CROP PLANTS IN THE MADRAS PRESIDENCY—II.

Estimation of Disease Resistance in Sugarcane Mosaic.

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Introduction.

Mosaic is a serious disease of cane in South India. In susceptible varieties the loss due to this disease is great but as the symptoms are not easily recognisable in the field to the untrained eye, the disease has been ignored by our cultivators so far. The existence of the disease in South India was first noticed in the year 1924 at Nellikuppam in the cane farm belonging to Messrs. Parry and Co. The firm suffered a heavy loss of crop owing to their bulk variety 'Red Mauritius' being affected with mosaic. This led to a rapid survey of the disease in the Presidency when it was found that it was widely spread in the cane-growing districts. As alarming reports of the loss of crop due to this disease had been received from other sugar-growing countries, the Government Mycologist of Madras, Mr. S. Sundararaman, took on hand the investigation of the disease in its varied aspects, and the testing of susceptibility and resistance of different varieties of cane to mosaic formed a part of the programme of this work. Since then, nearly 130 varieties have been tested and in the following paper are discussed the methods of work and results obtained, together with other relevant observations that were made during the course of the investigation.

Very few attempts have been made so far to assess the varietal susceptibility of canes to mosaic on a systematic and experimental basis. Lyons (1920) and Kunkel (1924) in Hawaii have recorded the range of susceptibility of a large number of varieties which were tested on a field scale. But beyond this all our knowledge on the subject has been based on stray and empirical observations of divers workers.

Materials and Methods.

It was found from observations of the cane plots in the Central Farm, Coimbatore, that secondary infection was taking place on a large scale and healthy clumps growing in the vicinity of diseased ones easily contracted

the disease especially during the first four months after planting. It was, therefore, found possible to lay out an experiment on a field scale by growing the varieties to be tested along with diseased canes side by side. The plan was as follows :—

A B A C A D A E, etc.

[A : Diseased material. B, C, D, E, etc. : Healthy material (varieties to be tested).]

Setts for planting the experimental rows were chosen from clumps selected with great care ; each clump being carefully examined for mosaic and only those that were found entirely healthy being selected for planting. A large sample of the material was also grown far away from the infected field as a check to see that the material chosen was really free from primary infection. The setts were planted in rows 4 feet apart in order to facilitate easy observation. In the alternate rows, setts cut from canes showing unmistakable symptoms of infection were planted and each variety to be tested was planted in at least 2 rows. As soon as the cane was well above ground, careful observations were made on each stool to see whether or not the plants showed mosaic. In the rows planted with diseased material, the symptoms of mosaic were discernible in the fourth or fifth leaf, *i.e.*, 14 days after planting and invariably 100 per cent. of the plants raised from diseased setts showed the symptoms, but in healthy rows the disease did not make its appearance in the very early stages, *i.e.*, till one month after planting. Observations with regard to the incidence of the disease as indicated by the leaf symptoms were made week by week. An accurate record was maintained of the number of infected canes in each variety. As it was found in practice, that in most susceptible varieties all the shoots springing from a clump developed symptoms sooner or later, plants showing symptoms even in a single shoot were marked as diseased. If, however, the other shoots did not in course of time exhibit the symptom, the fact was also noted. In varieties which showed some peculiarity of symptoms or in any other way found to be out of the normal, individual shoots and clumps were labelled separately and the progress of disease was carefully watched. As a check to the experimental rows, a few rows of healthy Co. 213 were grown and the progress of the disease in these rows gave an indication of the extent of secondary infection during the year. The percentage of infected plants to total in each experimental variety afforded an easy basis for evaluating the relative resistance of the variety.

Spread of infection.—During the first month after planting, infection spread gradually but in the third and fourth months infection was rapid,

the maximum percentage of infection in each variety being reached in about the fifth month ; no infection occurred after that period.

Though the fact of transmission by *Aphis maidis* was definitely established as early as 1920 by Brandes, considerable doubt existed in the minds of workers as to the exact mode of transmission under field conditions as *Aphis maidis* was seldom found to colonise on cane. In Coimbatore also the insect was found to colonise on cane permanently, but it colonised freely in the various grasses in the sugarcane field and also on crops like maize, cholam and ragi grown in adjacent fields and whenever these crops were cut or the field was weeded, large number of the alate forms of this insect migrated to the cane crop and rested for a considerable period on the spindle of the growing cane. Careful observation, extending for a period of 7 years, has shown that the migration is rapidly followed by the spread of the disease in the field.†

Testing of varieties by artificial infection.—In order to see whether the varieties which have escaped the disease in the field are really immune or not, a number of plants in each of these varieties were artificially infected by the pin-prick method.* It was found that only one plant in Co. 355 and one in Co. 205 took infection. Kassoer, P.O.J. 2878, Co. 335 and other resistant varieties did not take infection.

Susceptible stage of the plant.—The plants are susceptible to disease only in the early stages. When the crop is about 6 months old the danger of secondary infection is practically passed (*see* graph). This observation is of importance as it offers an easy means of controlling the disease by roguing out the diseased plants in the early stages when the elimination of a few clumps would not affect the yield to any appreciable extent.

Results.

The results of the experiments are summarised in Tables I and II in Appendix.

It will be seen that, with the exception of one or two useful varieties, most of the commercial varieties which have desirable characters are more

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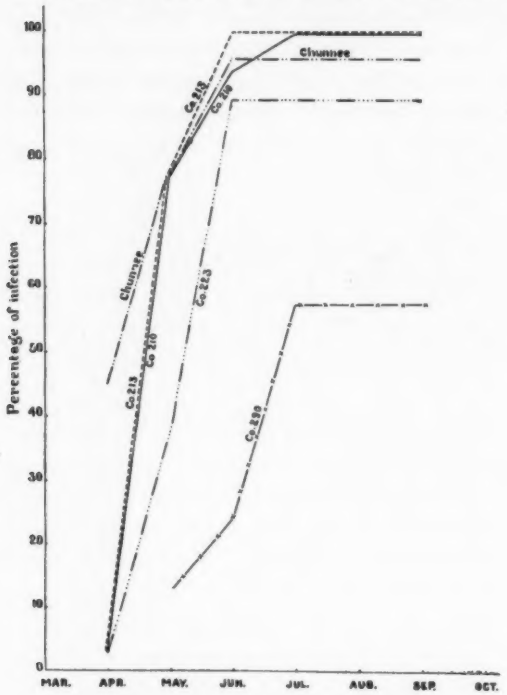
† The writer has been also successful in transmitting the disease through aphids under cage conditions.

* *Technique of the pin-prick method.*—A leaf from a mosaic-affected plant is tightly wrapped round the spindle of the experimental plant and a large number (100) of punctures are made with the help of a fine-pointed insect pin. This method gives the best results for artificial infection in Coimbatore.

Graph showing the progress of the Disease in the Varieties.



Date of Planting—1st week of March.

or less susceptible to the disease. For purposes of convenience, the results given in Table I may be discussed in groups.

Group I. Noble canes : Vellai, Poovan, Java Hebbal, Red Mauritius, B. 3412, B. 208 are highly susceptible. Fiji B, Fiji C are moderately resistant. P. O. J. 2878 is highly resistant. P. O. J. 2714, P. O. J. 2725 are also resistant to a moderately high degree.

Group II. Seedling canes : Co. 213, Co. 281, Co. 290, Co. 299, Co. 312, Co. 313, Co. 330, Co. 334 are highly susceptible. Co. 205 is resistant under Coimbatore conditions. Co. 214, Co. 244, Co. 315, Co. 316, Co. 335 are highly resistant.

Group III. Sorghum hybrids : Co. 354 is highly susceptible. Co. 353 is moderately susceptible. Co. 355 and Co. 356 are resistant.

Group IV. Seedling canes—Thick : Co. 413, Co. 412 are resistant. Co. 360 and Co. 361 are highly susceptible.

In assessing the relative susceptibility of the varieties as judged by the symptoms alone, it must be borne in mind that a large number of varieties which take the disease easily and may show symptoms of mosaic, are however highly tolerant and do not perceptibly suffer ill-effects, *i.e.*, their yield is not appreciably reduced. This is the case in some thin canes and also Fiji B among thick canes. But in other varieties, *e.g.*, Poovan, Red Mauritius, B. 3412 and B. 208 the plants get stunted in growth, and the diseased canes are markedly inferior to the healthy in size and weight.

In the course of the experiments, a few interesting observations were made with regard to the behaviour of certain varieties towards mosaic and they are recorded below.

Varieties Co. 360 *and Co.* 361.—The two varieties were peculiar in that the symptoms of mosaic in affected plants gradually disappeared as the plants grew older. It was thought at first that this might be a case of 'masking', but when setts from these were planted no mosaic symptom was discernible in the plants raised. These are possibly cases of genuine recovery. When these plants showing mosaic symptoms were cut and the ratoons were observed, no mosaic symptom could be noticed in the ratoon either. With a view to ascertaining if these recovered plants acquired any sort of immunity, a series of inoculation trials was done by means of the 'pin-prick' method.

1. From Diseased Co. 213 to recovered Co. 361 and Co. 360 plants.
2. From 361 Recovered plants to healthy Co. 213.
3. From Diseased Co. 361 and Co. 360 to Co. 361 and Co. 360.

No infection occurred in any of these cases and there is reason to believe that the recovered plants have acquired a sort of immunity against further infection.

Co. 205.—The behaviour of this variety under Coimbatore conditions is peculiar. It is reported to be highly susceptible in Bihar (McRae) and in the year 1930 it was found that nearly 10 per cent. of the 205 crop grown at the Cane Farm at Nellikuppam was diseased. But no mosaic has been noted so far in this variety either in the experimental plots or in the other cane areas at Coimbatore. In order to investigate the problem, setts of this variety were obtained from Pusa and plants were raised in the varietal resistance plots along with other canes. The results showed that while the first year crop showed about 4 per cent. disease, presumably due to primary infection, in subsequent years no disease could be noticed, thus definitely establishing the fact that Co. 205 is not susceptible to the disease under Coimbatore conditions. Three possibilities are suggested :

(1) That the environmental conditions at Coimbatore are not favourable for infection of the variety.

(2) The insect vector responsible for the spread of disease at Coimbatore is not able to transmit the disease on this variety.

(3) That different viruses are responsible for the disease at Coimbatore and Pusa and the variety is immune to one and susceptible to another.

The third possibility is ruled out because subsequent experiments showed that the disease can be transmitted easily to Co. 205 by the pin-prick method. With regard to the other two possibilities nothing can be said definitely at present.

Factors of resistance.—There appears to be no morphological differences between the resistant and susceptible varieties. Hand and microtome sections of leaves of four susceptible and four resistant varieties gave no clue whatsoever to any factor which was correlated with disease resistance.

Inheritance of resistance.—Since the ultimate ancestry of most of the canes mentioned in this paper are not definitely known to the writer, no attempt has been made to analyse the canes according to their parentage. It would appear that canes containing *Saccharum spontaneum* blood show resistance to mosaic to a greater extent than others.

Summary.

From the foregoing, it is seen that (1) great differences in susceptibility to mosaic are exhibited by the varieties of sugarcane, (2) that most commercial varieties are susceptible to this disease, (3) that some highly susceptible varieties are tolerant to the disease, (4) that a few immune varieties exist and could be made use of as parents, (5) that certain varieties which escape infection under one set of conditions may take the disease under other conditions, and (6) that though the disease is systemic, certain varieties recover from the disease and there is a possibility of the recovering plant acquiring permanent immunity.

Acknowledgement.

The writer is greatly indebted to Rao Bahadur Mr. S. Sundararaman, Government Mycologist, for guidance in the work and help in the preparation of this paper.

TABLE I.

Sugarcane Mosaic—Varietal Trials.

Table showing Percentage of Infected Plants in each Variety for the Years 1929–35.

Year—1929		1930		1931		1932	
Variety	%	Variety	%	Variety	%	Variety	%
Co. 205 Pusa	Nil	Co. 205 Pusa	2·5	Co. 205 Pusa	Nil	Co. 205 Pusa	4·0
„ 210	95·8	„ 213	97·5	„ 205	Nil	„ 205	Nil
„ 213	98·3	„ 214	Nil	„ 213	42·0	„ 213	100·0
„ 214	Nil	„ 244	Nil	„ 214	Nil	„ 214	Nil
„ 223	91·7	„ 290	61·0	„ 244	Nil	„ 244	Nil
„ 244	Nil	„ 299	76·5	„ 314	Nil	„ 270	19·5
„ 250	95·2	„ 312	85·3	„ 315	Nil	„ 285	31·0
„ 281	76·1	„ 313	98·5	„ 316	Nil	„ 291	92·5
„ 287	84·9	„ 314	1·3	„ 318	Nil	„ 300	98·0
„ 290	55·1	„ 315	Nil	„ 335	Nil	„ 301	67·0
Kassoer	Nil	„ 316	Nil	P.O.J. 2714	2·5	„ 310	Nil
Chunnee	96·6	„ 317	66·3	„ 2725	2·5	„ 314	Nil
P.O.J. 2714	1·6	„ 318	Nil	„ 2878	1·5	„ 315	Nil
„ 2727	9·3	„ 330	98·8	E.K. 28	23·0	„ 318	Nil
Pattapatti	60·0	„ 334	93·8	Fiji B.	17·0	„ 335	Nil
H. 109	Nil	„ 335	Nil	B. 208	66·0	Fiji B.	78·0
		P.O.J. 2878	Nil	„ 247 B.	23·5	B. 3412	100·0
		Kassoer	Nil			E.K. 28	100·0
		P.O.J. 2714	5·0			P.O.J. 2878	Nil
						„ 2727	28·0

TABLE I.—(*Continued.*)

Sugarcane Mosaic—Varietal Trials.

1933		1934		1935	
Variety	%	Variety	%	Variety	%
Co. 205	Nil	Co. 205 Pusa	Nil	Co. 205 Pusa	Nil
,, 205 Pusa	Nil	,, 205	Nil	,, 213	63·5
,, 213	95·0	,, 213	95·0	,, 229	Nil
,, 335	Nil	,, 335	Nil	,, 313	63·4
,, 360	73·0	,, 354	55·0	,, 326	Nil
,, 361	70·0	,, 355	Nil	,, 331	38·0
,, 363	39·0	,, 360	81·5	,, 335	Nil
,, 402	7·5	,, 361	55·5	,, 353	5·4
,, 408	31·0	,, 363	17·0	,, 354	55·2
,, 412	8·5	,, 402	13·0	,, 355	Nil
B. 3412	70·5	,, 408	13·5	,, 356	Nil
E.K. 28	85·5	,, 411	4·0	,, 360	55·4
Poovan	62·5	,, 412	10·5	,, 361	53·4
Vellai	79·5	,, 413	4·0	,, 363	22·7
P.O.J. 2878	Nil	,, 417	49·0	,, 402	13·4
		,, 419	43·0	,, 408	37·5
		P.O.J. 2878	Nil	,, 411	3·8
				,, 412	14·3
				,, 413	Nil
				,, 417	43·5
				,, 419	43·2
				,, 421	30·4
				,, 426	43·0
				,, 429	39·8
				,, 508	Nil
				P.O.J.2878	Nil
				Pundia	25·5
				Co. 515	54·2

TABLE II.

Statement showing Comparative Resistance to 'Mosaic' of Different Varieties of Sugarcane Tested for Disease Resistance in the Central Farm during the Years 1926–35. (*Under Experimental Conditions.*)

Resistant 0 to 5 % infection	Moderately susceptible 5 to 40 % infection	Varieties highly susceptible showing 40 to 100% infection under experimental condition
Co. 205 (CBE)	Co. 285	Co. 210
,, 205 (Pusa)	,, 270	,, 213
,, 214	,, 331	,, 223
,, 229	,, 353	,, 250
,, 244	,, 363	,, 281
,, 310	,, 402	,, 287
,, 314	,, 408	,, 290
,, 315	,, 412	,, 291
,, 316	,, 421	,, 299
,, 318	P.O.J. 2714	,, 300
,, 326	,, 2725	,, 301
,, 335	,, 2727	,, 312
,, 351	Fiji B	,, 313
,, 356	,, C	,, 317
,, 411	247 B	,, 330
,, 413	Pundia	,, 334
,, 508	Q 813	,, 354
P.O.J. 100	B 147	,, 360
,, 2878		,, 361
Kassoer		,, 417
		,, 419
		,, 426
		,, 429
		,, 515
		Chunnee
		Pattapatti
		H. 199
		B. 3412
		,, 6308
		D. 131
		,, 625
		,, 1135
		Java Hebbal.
		M. 55
		Red Mauritius
		Q. 166
		Butjan
		Vellai
		Poovan
		E. K. 28
		Striped Mauritius
		Tana
		B. 208
		B. 6450
		Purple Mauritius

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THE INHERITANCE OF MORPHOLOGICAL CHARACTERS AND OF WILT RESISTANCE IN REHAR (*CAJANUS INDICUS* SPRENG.).

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The property of disease resistance is generally physiological in nature and cannot be visibly identified by an observer in the same way as a morphological character. It happens sometimes that resistance may be closely associated with some definite visible morphological characters. Where such is the case the recognition of resistant types is comparatively easy.

In the case of the wilt disease of pigeon pea or *rahar* (*Cajanus indicus* Spreng.), the existence of resistance in certain pure lines has already been demonstrated. It has been shown that a high degree of resistance is present in the Pusa Type 80 and that resistance is also possessed by Types 16, 41, 50, 51 and 82 (McRae and Shaw, 1933).

The inheritance of wilt resistance as well as of other morphological characters in *rahar* was studied in a cross of Type 80 (resistant) × Type 5 (susceptible). The F_1, F_2 and F_3 generations were grown in artificially infected fields and the percentage loss due to wilt was determined. At the same time populations of F_1, F_2 and F_3 generations were grown in non-infected fields in order to compare phenotypic frequencies under the two conditions, and it was possible to determine whether or not resistance was associated with particular phenotypes.

It was found that resistance to wilt was inherited independently of flower colour, erect or spreading habit of growth, short or tall stature of plant crowded or open inflorescence and brown or grey markings of the seeds.

In Type 80 diffused red colour is present on the dorsal side of the standard while in Type 5 red colour is absent. In the F_1 form the cross between these two types, the dorsal side of the standard had faint veins while in the F_2 a new type, called " ringed " was produced. This double recessive type was characterised by the possession of red dots on the margins of the dorsal side of the standard. The erect habit of Type 5 was partially dominant to the spreading habit of Type 80. Monofactorial segregation was observed in the F_2 generation. The short stature of Type 5 was completely dominant over the tall stature of Type 80 plants and the F_2 generation segregated in the ratio of 3 short : 1 tall plants. The crowded

inflorescence of Type 5 also behaved as a simple dominant. The brown markings (patches) of seed of Type 80 were dominant to the grey dots of seeds of Type 5 on a monofactorial basis.

The inheritance of wilt resistance in *rahar* is illustrated by the data presented in Table I.

TABLE I.

Percentage loss due to wilt in F_1, F_2 and the parents.

Culture	1928—29 Branch plot 7	1929—30 Branch plot 8	1932—33 Branch plot 7	1932—33 Lawn plot 2	1933—34 Lawn plot 3
Type 8	7·3	47·5	18·6	2·2	14·0
Type 5	98·7	99·0	97·3	95·2	91·3
F_1 (T·80 × T·5) ..	8·3	..	26·5	27·9	
F_2 (T·80 × T·5) ..		*80·5 (93 : 385)	32·7 (191 : 93)	41·1 (158 : 106)	
F_2 (T·5 × T·80) ..			41·6 (101 : 72)	43·6 (119 : 92)	50·4 (508 : 516)

* The figures in parentheses show the proportion of resistant: susceptible plants from which the percentages were calculated.

It will be seen from the table that the greatest losses due to wilt occurred in Barah plot 8. The percentage losses in Type 80, Type 5 and F_2 generations were 47·5, 99·0 and 80·5 respectively. This was apparently due to the heavy infection of the soil with the wilt organisms. This plot had been under experiments dealing with the *rahar* wilt for the four successive seasons and the wilt organism had gradually become abundant. Where this is the case even resistant types may be considerably attacked (McRae and Shaw, 1933).

With the exception of the figures in column 3 (Barah plot 8), the data indicate that probably two complementary factors are involved in the inheritance of wilt resistance. Out of 1,956 F_2 individuals 879 were susceptible and 1,077 resistant to wilt. The deviation from the figures expected on a ratio of 9 resistant : 7 susceptible is $23·25 \pm 14·85$ and is quite within the limits of chance deviation. However, it must be pointed out that the validity of such data depends upon the uniformity of the artificial infection. If such uniformity is not attained, some of the susceptible plants may escape infection and consequently be classified in the resistant group to which they do not belong.

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THE CONTROL OF FUNGAL PARASITES BY THE PLANT.

By L. D. Galloway.

(*From the Imperial Institute of Agricultural Research, Pusa.*)

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I have only a very general contribution to offer to this discussion, with a few illustrations and suggestions relating to fungus control by the plant.

From the severely practical point of view the question of disease resistance has resolved itself into an attempt secure—by selection or hybridisation or importation—plants resistant to troublesome diseases, without worrying as to how this state is achieved.

The actual mechanism of the factors leading to disease resistance should not, however, be regarded as a purely academic enquiry. Further results in this direction can hardly fail to include some which are capable of a practical application, even if only in "speeding up" the sorting tests of new varieties.

In the long run, of course, the true test of resistance is behaviour in general agricultural practice over an extended period.

Most problems regarding the control of fungi by human agency—including the fungi that do so much damage on foodstuffs and industrial products such as textiles—may be conveniently dealt with by considering some such scheme as the following, which lists the chief essentials for the development of these organisms :

I. Infection.
II. Humidity.
III. Nutrition.
IV. Temperature.
V. Atmosphere.

This scheme will also serve quite well for classifying the methods at the disposal of the plant and as a framework for a few random remarks and suggestions regarding the investigation of disease resistance.

I. Infection.

I have put *infection* first on the list since one obviously cannot have fungal growth without a fungus. One of the stock tricks for escaping

certain plant diseases is of course to use varieties with a different sowing time or different time of ripening, so that the life-histories of the host and parasite are, as it were, put out of step.

Infection may be seed, soil, or air-borne. As regards the last, the extent, shape and nature of leaf surface will of course influence the number of spores settling on the plant and hence the potential infection.

Avoidance of insect infection may also be included under this heading. Many fungal diseases, *e.g.*, of sugarcane, are conveyed by insects or are only able to establish themselves on tissues damaged by insects. Unpalatability to insects is therefore a valuable asset in securing resistance to fungal attack.

For the sake of convenience one may also consider under this heading of Infection, those cases where the plant reacts to wounds or fungus attack by the production of corky protective layers, or the exudation of gum, thus shutting off further infection from the damaged tissues.

II. Humidity.

This is perhaps the most important single factor, and a rise in humidity often leads to a sudden onset of disease where none was seen before. A very striking example of this occurred recently at Pusa when after a few wet days sann-hemp seedlings showed a very heavy mortality due to attack by *Colletotrichum curvatum* (the first record of this fungus in India) ; seed-beds of the same varieties planted a week or two earlier, had shown no trace of the disease. Water-logging of the soil has also been shown to cause a predisposition to attack by various root fungi.

Little has been recorded as to the minimum moisture requirements for spore germination. I recently had occasion to determine this for about 40 fungi, mainly saprophytic moulds but including a number of semi-parasitic types. I found that the moisture requirement was best considered in terms of the relative humidity of the atmosphere and was independent of the moisture content of the substratum when this was in equilibrium with the atmosphere. Two materials kept in an atmosphere of 90 per cent. R.H., for example, might take up moisture to the extent of 10 per cent. and 20 per cent. respectively, but the moisture available to the fungus was the same in each case. Spores of certain types of *Aspergillus* and *Penicillium* could germinate at R.Hs., as low as 75 per cent., although most required 80–85 per cent. The facultative parasities tested, including species of *Cladosporium*, *Thielaviopsis*, *Alternaria* and *Acrothecium*, required humidities of 90 per cent. or slightly more.

Rust spores have been shown to require saturation humidity or even liquid water for germination, and it is probable that the majority of plant

parasites will be found to require a humidity of 95–100 per cent. Little has been recorded in this direction for the vast range of parasitic fungi lying between the facultative parasites and the highly specialised types such as rusts, but a recent unpublished determination for *Colletotrichum graminicolum* gives the minimum R.H. as 95 per cent.

Humidity conditions can be, to some extent, modified by man, but the plant is of course largely at the mercy of climate. Such factors, however, as the retention of water or water vapour by hairy surfaces, rosette leaves close to the soil, or enclosed areas of plant organs cannot fail to produce an effect on the ease with which spore germination occurs.

Further work on the moisture requirements of parasitic fungi is needed, and will be found to bear on the question of plant resistance.

III. Nutrition.

This is the most complex of our five factors. What is it that makes so many parasitic fungi incapable of growing on artificial media? At Pusa, we recently carried out a few experiments in adding sterile unheated extracts of the host tissues to an agar medium, but none of the fungi tested showed any increased inclination to develop. Then again, why do some fungi parasitise a variety of hosts, whilst others are restricted to a single species? And why is a devitalised plant susceptible to infection? Some of these differences may be associated with the nature of the food supply of the parasite, *i.e.*, the plant tissues, and attempts have been made to correlate them with differences of *p*H—usually rather unconvincing in magnitude—or with some other measurable factors.

Different soil constituents and fertilisers often affect resistance, either by some effect on the composition of the plant tissues, or by affecting the vitality of the plant. In general, the addition of phosphates is claimed to increase resistance, whilst addition of nitrogenous substances decreases it. This does not always follow; for example, the wilt of pigeon pea is reduced by the use of green manure but increased by addition of superphosphate.

Although there is nothing quite analogous to blood circulation in plants, there seem to be similar possibilities of "toning up the system" or whatever the medical phrase of the moment may be; and converse possibilities of increasing susceptibility by "under-nourishment", "wrong feeding", etc. One day perhaps we shall be able to express these matters in more biochemical language, and it will be possible, though not always feasible, to give to a diseased plant a tonic, or an internal antiseptic, introduced in some suitable manner. In fact, some progress has already been made on these lines by various workers, in India as well as in other countries.

Continuing the analogy with animal diseases one is led to consider the possibility of immunising plants to certain diseases. So far results in this direction have been disappointing. In some cases (tobacco root-rot, cotton wilt) disease symptoms may be produced by treating the plant with a toxic extract obtained from a culture of the parasitic fungus. The use of controlled doses of this type should be helpful in future biochemical research on disease resistance.

Under the heading of nutrition one may also consider the action of poisons. The higher plants and the fungi show a very regrettable similarity in their susceptibility to poisons. Thus although many plants produce substance toxic to insects and animals, these substances rarely produce any marked inhibiting effect on fungal growth. Bactericidal substances are known, as in hops, but bactericides are not always fungicides, and hop mildew is quite unabashed by the presence of this constituent.

There is, however, often a narrow margin between toxicity to plant and to fungus, such as that which enables us to make use, for example, of slightly soluble copper salts as protective sprays. We may expect to find that certain cases of plant resistance are linked up with the presence of phenolic plant products of low toxicity.

In most cases, the reaction of host and parasite is of a more elusive nature, some mysterious battle of the enzymes deciding whether the fungal invader kills the host cells or the host cells kill the parasite. So little is known of the reactions involved that progress in this direction is likely to be slow for the present, but this is really the crux of the whole problem of plant resistance.

IV. Temperature.

Temperature is of course a factor of considerable importance, both in its action on the growth of the parasite and on the resistance of the host. It is not however a factor that the plant can make use of to any great extent. In some cases the resistance that one is apt to put down to temperature differences may be due to a combination of climatic and soil factors. *Tilletia indica* which causes bunt on wheat in the Punjab, seems quite incapable of developing under Pusa conditions. *Sclerotium oryzæ* which causes a serious stem-rot of rice in some countries, is capable of development under Pusa conditions, but fails to produce any disease symptoms. In these cases temperature may or may not be the deciding factor. The maize smuts and late blight of potato occurring in the hills but not in the plains of India, are almost certainly controlled mainly by temperature. A still clearer case noticed during recent experiments at Pusa is the "black shank" disease

of tobacco seedlings, which cannot be induced at all during the winter months, but can be made to cause 100 per cent. deaths from April to October.

V. Atmosphere.

Most fungi require an adequate air supply for growth. Recent work has however shown that growth may be checked by certain additions to the atmosphere. Carbon dioxide in concentrations exceeding 10 per cent. begins to exert an inhibiting effect—a fact whose utilisation now enables chilled beef to be transported from Australia to Europe. In the case of ammonia, acetaldehyde and certain other volatile substances, in an extremely minute amount in the atmosphere, is sufficient to check fungal growth. Even naphthalene, put in herbarium cabinets and book cases to prevent insect attack, serves the additional useful purpose of checking growth of mould fungi. Thymol is still better for this purpose.

It has been found that the outer scales of certain onion bulbs contain a volatile product which checks the growth of most fungi. Further research will probably show that many of the aromatic substances and essential oils of plants exert a similar protection active which plays a part in the prevention of disease.

* * * * *

In conclusion, the mechanism of disease resistance presents a fresh series of problems for each host plant and each parasite, and no general formula is to be anticipated. It may perhaps be emphasised also that this type of research offers unrivalled opportunities for jumping to over-hasty conclusions. A closer co-operative study of the subject is however to be heartily welcomed, especially co-operation by biochemists and physical chemists, who have not tackled this question very seriously so far, and whose findings cannot fail to give a stimulus to further biological research.

RESISTANCE OF AMERICAN COTTONS TO FUSARIUM WILT IN INDIA

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Introduction

The high resistance bordering on complete immunity possessed by the Indo-American cottons (*Gossypium hirsutum* Mill.) to *Fusarium* wilt in India was noted even as early as 1908 by Evans (1908). On the Nagpur Experimental Farm where he first recorded *Fusarium* wilt, not a single American-Upland cotton plant was affected while mortality in the indigenous cottons was rather high. Ajrekar and Bal (1921) noted the same fact and Kottur (1924) confirmed these observations from his experience in the Southern Mahratta Country.

During a period of eight years when the writer was associated with the cotton wilt investigations at Dharwar not a single case of wilt in this cotton came under his notice even though several acres of it were being grown year after year in the wilt-sick fields on the Dharwar Agricultural Farm. Kulkarni (1934) reporting the relative resistance of various cotton varieties grown on the wilt-plot at Dharwar has shown that in the eight years from 1924 to 1932, the Indo-American cotton known as Gadag I was never attacked by the wilt disease while the more recent introductions from America like Sea Island, Cleveland Big Boll, Dixie Triumph and several others remained equally immune.

This could be explained on the assumption that parasitic specialisation had so far progressed in *Fusarium vasinfectum* Atkinson, the causal organism of wilt, that the Indian form was unable to attack foreign varieties of cotton. In 1925 a culture of a virulent strain of the American fungus was therefore obtained from Dr. V. H. Young of the Arkansas Agricultural Station and used in pot experiments at Dharwar with both sterilised and unsterilised soil. These tests showed that even this American strain was quite incapable of attacking both the Indo-American and the Indian cottons. Even the recently introduced susceptible variety like Cleveland Big Boll had acquired an immunity to its own pathogen under Indian conditions.

Tests with the American strain of the parasite on American and Indian cottons were continued in 1926 and 1927 with confirmatory results and the

explanation for the high resistance shown by American and Indo-American cottons to *Fusarium* wilt under Indian conditions had to be looked for elsewhere. Presumably some factor of environment operated which made either the pathogen incapable of attacking its host or made the host resist the invasion by the parasite.

Experimental

According to Atkinson (1892), Orton (1900), Fulton (1907) and others, cotton wilt is prevalent in America on light, sandy soils that are acidic in reaction. Taubenhaus, Ezekiel and Killough (1928) state that sixty-three per cent. of the fields where wilt is very common in the United States have soils with a reaction ranging from 5·5 to 5·9 pH. In India, however, cotton wilt is confined to the black cotton soils which are stiff clays with a reaction ranging from 7·6 to 8·0 pH. Both the texture of the soil and its reaction had presumably operated in rendering the Indo-American cottons highly resistant even to their own pathogen.

An opportunity to test the parasitism of the Indian *Fusarium* to Indo-American cottons in acidic soils and the influence of the sandy and acidic American soils on the infectivity of the Indian fungus to Indian cottons presented itself in the summer of 1929 when the writer was located at Ames, Iowa, in the U. S. A.

The Indian strain of the fungus and the Indian (Dharwar I) and the Indo-American (Gadag I) cotton seed were obtained from Dharwar and a culture of the American fungus was secured from Dr. V. H. Young. Ames is not in the cotton belt and the soil for these experiments was therefore obtained through the courtesy of Dr. G. Jensen of the Arkansas Agricultural Experiment Station from a typically wilt-infested plot in the Cotton Research Station at Marianna. Half this soil was distributed into eighteen six-inch pots and these were sterilised together with the soil at 35 pounds pressure in the autoclave. The rest of the soil was distributed into twenty pots of same size but the soil was not, however, sterilised.

Giant cultures of the Indian and the American strains of the parasite were made on sterilised barley and a weighed and a constant quantity of the inoculum was added to the respective pots. Care was taken to see that the Indian and the American fungus material did not get mixed up and every time the pots were handled, the hands were washed with mercuric chloride solution. In seven pots that had unsterilised and wilt-sick soil, the American strain of the fungus was introduced to ensure infection. In these pots the parasitism of the American fungus to Indian cottons was tested. The results are recorded in Table I.

TABLE I

Tests with Indian Fusarium *in American Soil*

Cotton variety	Strain of fungus	Pots	Plants	Plants wilted	Remarks
Indian (Dharwar I) ..	Indian	7	28	6	Soil sterilised
Indo-American (Gadag I)	Indian	8	32	0	do.
Indo-American (Gadag I)	No fungus	3	12	0	do.
Indian (Dharwar I)* ..	American	7	28	0	Unsterilised
Indo-American (Gadag I)	American	7	28	23	do.
Indian (Dharwar I) ..	American	6	23	0	do.

* These seven pots received additional infestation.

The data recorded above show that the Indian fungus is very slightly pathogenic to Indian cottons in the sandy and acidic American cotton soils. It is, however, unable to attack American or Indo-American cottons either in American soils or Indian soils. Neither is the American fungus able to attack Indian cottons under any circumstances.

Discussion

From the observations reported above, it can be inferred that the factors of environment play a very decided rôle in the pathogenicity of parasitic fungi and the resistance which the host plants can offer to them. A virulent parasite like the American strain of *F. vasinfectum* can apparently become a harmless saprophyte to its susceptible host when the latter grows in a soil with a different texture and reaction. Whether the effect of the environmental factor is on the host or the parasite or both, it is difficult to say. A virulent strain of the Indian *F. vasinfectum* evidently gets much attenuated also in its parasitism towards its Indian host when the latter is growing in the light, sandy and acidic soils of the United States. These experiments further demonstrate that in addition to the factors of environment, the parasitic specialisation within the species of a pathogenic fungus is also a factor that has to be taken into consideration in accounting for host resistance. The Indian strain of *F. vasinfectum* is a distinct physiologic race that is unable to attack American cottons and similarly the American strain is unable to attack Indian cottons. Fahmy (1927) reports that specialisation of the parasite occurs in Egypt, the Egyptian form of *F. vasinfectum* being, however, mildly pathogenic both to the Indian cottons and the American cottons.

The tests have further brought out that even though the American cotton has been acclimatised in India for the past one hundred years and

more and has been growing away from its parasite for such a long time, yet, it has not acquired any resistance to that fungus. When such cotton was brought into contact with its parasite under proper conditions, the plants succumbed to the disease.

Summary

1. It is a well-known fact that Indo-American cottons are immune to *Fusarium* wilt in India.

2. These cottons as well as the recently introduced American varieties also proved to be immune to an American strain of the fungus when growing in Indian soils.

3. The Indo-American cottons were tested in America on American cotton soils and there they proved to be as susceptible as the American cottons to the American strain of the fungus, but they were still immune to the Indian fungus.

4. The American and the Indian strains of *F. vasinfectum* show, therefore, a very strict parasitic specialisation, the American form being unable to attack Indian cottons in any circumstances, and *vice versa*.

5. Indian cottons grown on American cotton soils are much less susceptible to the Indian form than when they are in India.

6. American cotton soils where wilt is common, are light, sandy and acidic but the Indian cotton soils where wilt is predominant are heavy, clayey and alkaline. The above experiments strongly suggest that the reason the Indian *Fusarium* weakly attacks its Indian host in American soils and the American *Fusarium* is almost non-pathogenic to its American and Indo-American host in Indian soils, is to be sought for in the fact that these soils have different texture and reaction.

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STUDIES IN DISEASE RESISTANCE—I COTTON WILT AND ENVIRONMENT.

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1. Introduction.

The wilt disease of cotton (*Fusarium* sp.) has been studied extensively by Ajrekar and Bal (1921), Fahmy (1928), Kulkarni and Mundkur (1928), Dastur (1929), Dharmarajulu (1932), Fikry (1932) and Kulkarni (1934). The last author has fully reviewed previous work.

In these studies, detailed attention has been given, mainly, to the behaviour of the causal organism under (1) controlled laboratory conditions or (2) in the fields. All the observations on the environment and the host have been made in order to understand the nature or the mechanism of the activity of the fungus.

There are, however, a few observations here and there, suggesting the possibility of other factors operating.

The following points are mentioned by Dastur (1929):

(1) The initial symptoms are the same, both in wilt-affected plants as well as mature healthy plants growing in diseased soil.

(2) *Fusarium* hyphæ may not be present in the wilt-affected plants.

(3) Superphosphate seems to have some residual effect on disease.

(4) Incidence of wilt is influenced by the relative position of wilt-affected and healthy soil-layers in pots and also by the size of the pot.

(5) In healthy soil, infection may not develop wilt.

(6) Uninoculated healthy stems mixed with soil, may produce disease.

(7) Wilt can be produced in the healthy soil by non-pathogenic agencies, such as organic manure, aluminium salts, lime and water-logging.

(8) Seedlings in the very first stages of wilt-infection, if transplanted into healthy soil, develop into healthy mature plants.

(9) Aluminium salts are present in the tissues of wilt-affected plants and the discolouration due to its presence may extend further in plant tissues than fungal hyphæ.

Young (1928) found wilt more severe in the lighter soil of the United States of America, but Fikry (1932) mentions it to be more severe in the clay soils of Egypt. Rast (1932) attributes to potash the protection from wilt, observed by him from applying to very fine sandy alluvial loam, a mixture, at the rate of 1000 lb. per acre, containing 5% available phosphoric acid, 1·5% nitrogen and 6·25% potash. Young, Janssen and Ware (1932) and Maddux (1933) have also recorded similar observations. Fahmy (1928) and Dastur (1929) report an increase, while Kulkarni (1934), a decrease in cotton wilt with organic manure. The manure used by Dastur was unrotted. Fikry (1932) found an increased wilting in soils of high water-holding capacity and pH, 7·8 to 8·3, a reduction by light and no effect from increasing the CO_2 content of the atmosphere. Increased wilting with higher soil moistures has been noted by Johnson (1921) on tobacco, and with low moistures by Clayton (1923) on tomato, and by Tisdale (1923) on cabbage. Wardlaw (1930) associates banana wilt with bad aeration and death of roots. Greater compactness in subsoil, irrespective of soil texture and wider C/N ratio have been recently mentioned in connection with cocoanut wilt (1933). Whether the plant plays any part outside its inherent susceptibility or resistance in determining the extent of wilting, does not seem to have been mentioned anywhere. An investigation was started at the Institute in 1931 to secure fuller information regarding the nature and the influence of conditions—favourable and unfavourable to wilt—on the normal functioning of the cotton plant, and its effect on the activity of the fungus.

The preliminary work done is summarised below.

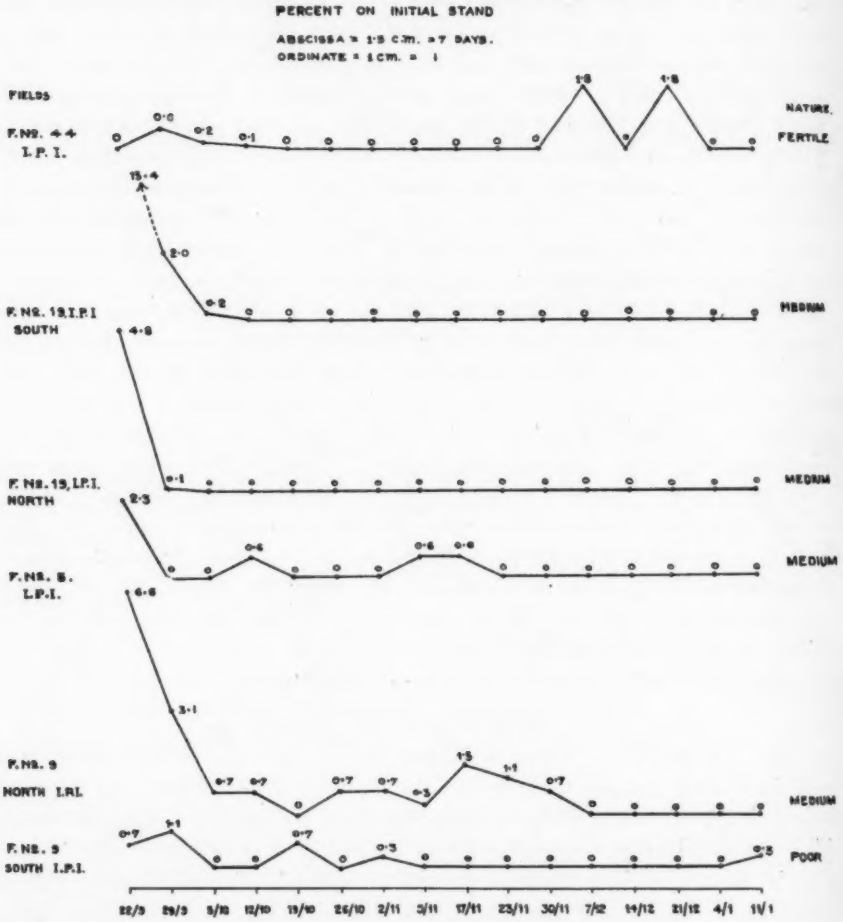
2. *Investigations and Results.*

(*a*) *Occurrence.*—Cotton wilt has never been considered a serious pest in Malwa, though there is no data recorded either to prove or disprove this. Scattered and patchy wilting was however seen in fields. A rough idea was obtained in the year 1933, of the nature and extent of wilt, occurring in fields of different grades of fertility, and at different intervals. Percentages of deaths on initial stand are given in Fig. 1. All types of wilt, mentioned by previous workers were found to exist. The mortality was low. Persistance of wilt during the season differed from field to field, irrespective of the initial intensity which probably represented the degree of infection at start. Portions of fields showing high incidence were marked for closer study.

(*b*) *Extent and periodic fluctuations.*—In order to ascertain the extent of the mortality in cotton plants, and its progress during the season in wilt-affected areas in Malwa, periodic observations on numbers of healthy and

FIG. 1

COTTON WILT IN FIELDS AT INDORE 1933.


PERCENT ON INITIAL STAND
ABSCISSA = 1·5 C.m. = 7 DAYS.
ORDINATE = 5 C.m. = 1
FIELDS
F. NO. 44 I. P. I.
NATURE.
FERTILE
F. NO. 19 I. P. I SOUTH
MEDIUM
F. NO. 19, I.P.I. NORTH
MEDIUM
F. NO. 8. I.P.I.
MEDIUM
F. NO. 9 NORTH I.P.I.
MEDIUM
F. NO. 9 SOUTH I.P.I.
POOR
22/9 29/9 5/10 12/10 19/10 26/10 2/11 9/11 17/11 23/11 30/11 7/12 14/12 21/12 4/1 11/1

wilted plants were taken during the years 1933–34 and 1934–35 in two Institute fields, 19 and 11 B, and in 1934–35 in field 11 C. Cotton was grown after cotton, during this period to intensify the existing infection. In addition, 600 lb. of a mixture of chopped cotton stalks and half-rotted compost infected with growths of *Fusarium* species was intimately mixed with the soil by ploughing in and *bakharing* the fields. The organism used was isolated from wilted cotton plants collected in the fields and grown in Richard's solution. Growths on 200 gms. of chopped cotton stalks steeped

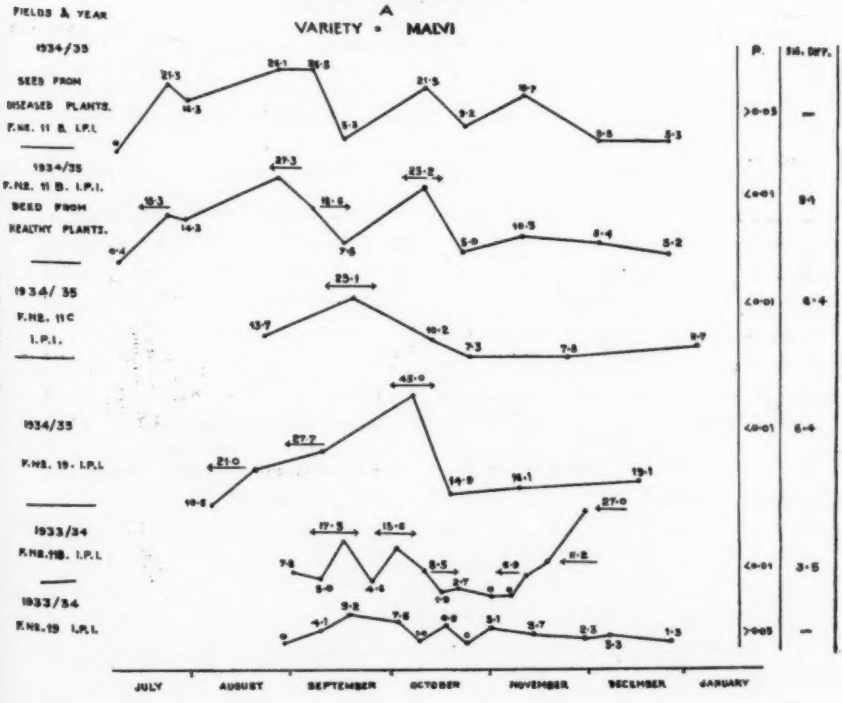
in 200 cc. of Richard's solution for fifteen days were mixed with twice the quantity of half-rotted compost, which was kept moist at monsoon temperatures (25° C.–27° C.) in the pot-culture house for eight days with occasional stirring. Both when mixing with compost and using in the field, the inoculum was examined for the presence of the fungus.

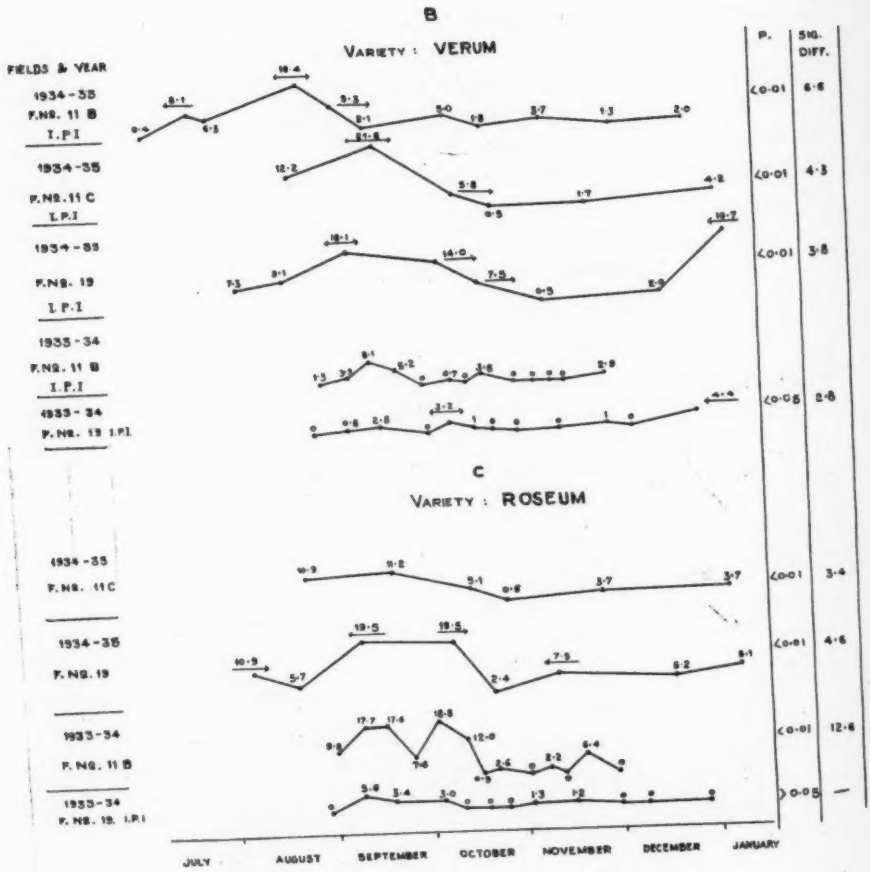
The local Malvi cotton (seed collected from both healthy and wilted plants), as well as the susceptible Roseum and Dharwar 1 and the resistant verum 262 and Dharwar 2, varieties were sown. Percentage deaths calculated on initial stand for each period are plotted in Fig. 2 A.B.C. with their statistical valuations. Numbers significantly higher than both the preceding and following ones, are marked with a double arrowed line ; and those significantly higher than an adjacent number with an arrow pointing one way.

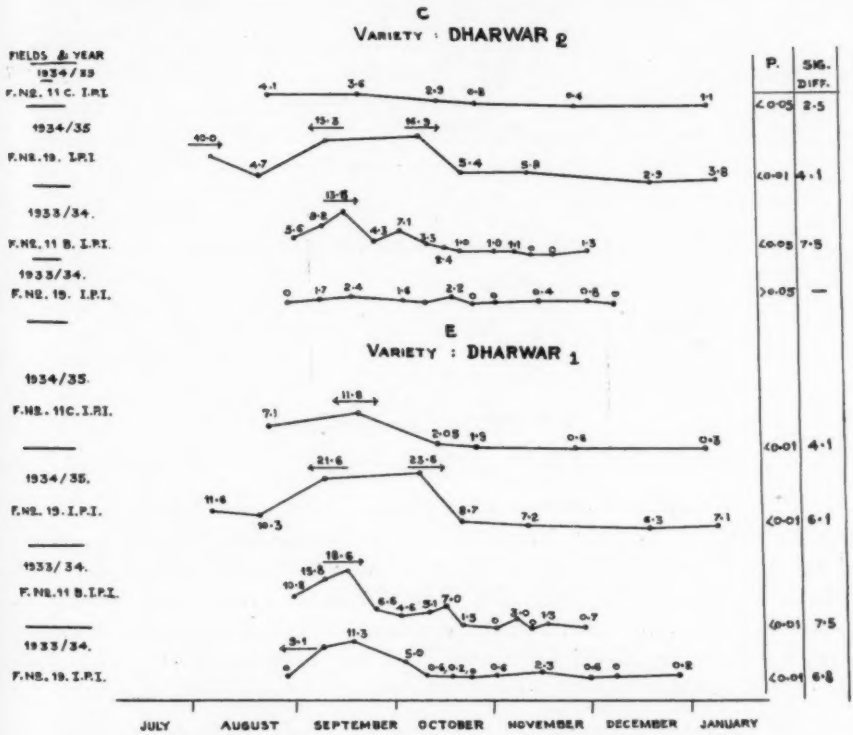
FIG. 2

PERIODIC FLUCTUATIONS IN WILTING.

PERCENTAGE OF DEATHS ON INITIAL STAND FOR EACH PERIOD.




B
VARIETY : VERUM
FIELDS & YEAR
1934-35
F.NO. 11 B
I.P.I
1934-35
F.NO. 11 C
I.P.I
1934-35
F.NO. 19
I.P.I
1933-34
F.NO. 11 B
I.P.I
1933-34
F.NO. 19 I.P.I
P.
SIG. DIFF.
<0·01 6·6
<0·01 4·3
<0·01 3·8
<0·05 2·8
C
VARIETY : ROSEUM
1934-35
F.NO. 11 C
1934-35
F.NO. 19
1933-34
F.NO. 11 B
1933-34
F.NO. 19 I.P.I
<0·01 3·4
<0·01 4·6
<0·01 12·6
>0·05 —
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY



It may be noted that (1) periodic fluctuations in death-rate may not vary significantly throughout the season ; (2) death-rate may gradually fall to a significantly lower level as time advances ; or (3) the death-rate may reach once or oftener, a significantly higher level than that of either the preceding or succeeding period, or of both, in different fields or season. Different varieties may not always behave similarly under the same conditions. A gradual fall without a peak was shown only by Dharwar 2 and Roseum. Dharwar 1 did not show more than one peak. Both resistant and susceptible varieties showed these environmental influence.

Irrespective of season and field, Dharwar 1 showed a greater susceptibility than Dharwar 2, but there was no such difference between Verum and Roseum (Table I).

TABLE I.

Mean percentage deaths, due to wilt, of resistant and susceptible varieties grown at Indore, irrespective of field and season.

	Varieties			
	Verum	Roseum	Dharwar 2	Dharwar 1
Mean % death	4·8	6·1	3·7	6·1
	$P > 0·05$		$P < 0·01$ · Sig. differ. = 1·8	

Thus it appears that a variety, which is commonly classed as " resistant " may be so only under certain environmental conditions. When those conditions are changed it may become susceptible, while under those identical changed conditions the " susceptible " variety may become resistant. The average mortality from wilt at the end of the season at Indore was found to be 63·4% for Malvi, 52·7 for Roseum, 50·4 for Verum, 31·1 for Dharwar 1 and 20·6 for Dharwar 2 respectively.

(*c*) *Temperature, humidity and light.*—Most of the deaths occur within the period when the soil temperature at 6 inches depth was between 25° C. and 22° C. and at 15 inches between 25° C. and 24° C. The temperatures fell later, as also the death-rate. This agrees with Kulkarni's observations in general.

Within this period a sudden rise in death percentage seems to coincide with the sudden rise in mean duration of sunlight, at the end of the monsoon, which rose from two hours to five hours daily.

In the later period, when the soil temperatures were low, duration of sunlight made no difference.

(*d*) *Soil conditions associated with wilt.*—The observed modifying influence of field differences on cotton wilt, suggested the examination of soils around the roots of healthy and wilted plants.

Total and hygroscopic moistures were determined in two zones around the roots of healthy and wilted plants during August, September and October of 1934. The results are given in Table II A.B.C.

TABLE II.

Mean percentage moistures in soil, around the roots of healthy and wilted cotton plants.

A. Total moistures.

Depths		Healthy plants	Wilted plants
3″—6″		27·7	35·1
9″—12″		29·2	30·3
		P	Sig. difference
Conditions	..	< 0·05	3·6
Depths	.. ..	> 0·05	..
Interaction (depth and conditions)		> 0·05	..

		Mean moisture percentage for both depths
Conditions	Healthy	28·4
	Wilted	32·7

B. Hygroscopic moistures.

Depths		Healthy plants	Wilted plants
3″—6″		9·7	12·9
9″—12″		11·6	10·8
		P	Sig. difference
Conditions	..	> 0·05	..
Depths	.. ..	> 0·05	..
Depths × Conditions	..	< 0·05	2·7

C. Ratios of mean percentage moistures for two depths around the roots of healthy and wilted cotton plants.

Ratio	Healthy plants	Wilted plants	P	Sig. difference
$\frac{9''-12''}{3''-6''}$	1·05	0·90	$< 0·05$	0·14

The moisture contents of soil around wilted plants were higher than around healthy plants. Ratios of moisture contents in the lower zones to those in the higher zones were greater than unity and higher for healthy plants than for wilted plants, for which the ratios were less than unity.

Hygroscopic moistures of the upper soil zones were higher around wilted plants than around healthy ones.

The physical condition of the soil around healthy plants seems to permit a greater percolation of water to lower depths, and conversely the upper zones around wilted plants are charged with larger amounts of water, because the soil has become less permeable (as indicated by the higher hygroscopic moisture of those zones).

(*e*) *Root length, its distribution in the soil profile.*—The extent and distribution of plant roots is influenced by differences in soil conditions (Weaver, 1926); hence the roots of healthy and wilted plants were examined to see if they showed any consistent differences.

The following method was used :—A trench was dug alongside the plants and the root system in a vertical plane exposed with a gentle spray of water from a jet of suitable size (Howard, 1929). Root-lengths were recorded in three-inch horizontal zones and further differentiated into dormant (living and capable of producing new roots), active (white coloured and tender), and dead (brownish or dark, apparently in different stages of decomposition). These were sketched on a scale of three to one, dormant roots tinted black, active red and dead green (Plate XXII).

Determinations were made on both healthy and wilted plants of (1) total root lengths and (2) the mean percentages on total, of the active* and dead root lengths, (3) Ratios of active to dead root lengths, (4) distribution of these in the depths 0–9, 9–18, 18 inches and below for two periods—August and September and October to December—in 1933 and 1934. The

* This is hereafter referred to as the "activity".

results of the examinations of eight pairs of root-washings with their statistical valuations are given in Table III.

TABLE III.

A. Distribution in soil profile of mean root-lengths per plant.

Periods	Healthy plants			Wilted plants		
	0″—9″	9″—18″	18″ & below	0″—9″	9″—18″	18″ & below
I August—September	129·1	79·9	34·5	70·6	43·1	20·5
II October—December	73·7	30·3	32·6	75·3	24·5	19·4

	P.	Significant difference
Conditions	< 0·05	19·3
Depths	< 0·05	70·8
Periods	< 0·05	57·9
All interactions	< 0·05	..

Conditions		Depths			Periods	
Healthy	Diseased	0″—9″	9″—18″	18″ & below	I August—September	II October—December
189·9	126·6	87·2	44·5	26·7	188·1	127·8

B. Distribution of mean percentages on total, of active root-lengths of healthy and wilted plants—1933 *and* 1934.

Periods	Healthy plants			Wilted plants		
	0″—9″	9″—18″	18″ below	0″—9″	9″—18	18″ below
August—September	30·2	19·1	38·5	5·1	15·8	27·0
October—December	20·2	20·9	57·4	7·0	5·5	19·2

	P.	Significant difference
Conditions	< 0·05	10·4
Depths	< 0·05	12·7
Periods	> 0·05	..
All interactions	> 0·05	..

Conditions		Depths		
Healthy	Wilted	0″—9″	9″—18″	18″ below
31·0	13·2	15·6	15·3	35·5

C. Distribution in soil profile of mean percentages on total, of dead root-lengths of healthy and wilted plants.

Periods	Healthy plants			Wilted plants		
	0″—9″	9″—18″	18″ below	0″—9″	9″—18″	18″ below
August—September	10·8	16·9	12·3	26·4	21·5	8·5
October—December	38·3	15·6	7·0	74·8	73·9	45·7

	P.	Significant difference
Conditions	< 0·05	12·9
Depths	< 0·05	15·8
Periods	< 0·05	12·9
Conditions × Periods	< 0·05	18·2
Other interactions	> 0·05	..

Conditions		Depths			Periods	
Healthy	Wilted	0″—9″	9″—18″	18″ below	I August—September	II October—December
16·9	41·8	37·6	32·0	18·4	16·1	42·6

Conditions × Periods.

Periods			
I August—September		II October—December	
Healthy	Wilted	Healthy	Wilted
13·3	18·8	20·3	64·8

D. Ratio (Active/Dead); root-lengths of healthy and wilted plants.

Periods	Healthy plants			Wilted plants		
	0″—9″	9″—18	18″ below	0″—9″	9″—18	18″ below
I						
August—September	2·79	1·13	3·13	0·19	0·73	3·17
II						
October—December	0·52	1·33	8·20	0·01	0·074	0·42

Total active root-lengths / Total dead root-lengths	For healthy plants	For wilted plants
	1·84	0·31

The total root-length was greater in the first period than in the second and decreased with depth in each period for both healthy and wilted plants. It was greater for healthy than wilted plants under all conditions.

The activity was always greatest below 18 inches, but equally distributed above. There was no difference in the activities observed in the two periods. Activity of healthy plants was greater than wilted plants for both periods and in all depths. For both healthy and wilted plants, the root death-rate was highest in the uppermost zone, fell in each zone below and increased in the second period. Wilted plants showed a higher root death-rate than healthy ones in each zone and period. This increase was so high that the root death-rate of wilted plants compared with that of healthy ones during the first period, was similar to that for healthy plants between the first and second periods.

The ratio of active to dead root-lengths was in general much greater than unity for healthy plants and less than unity for wilted, but when ratios for individual zones and periods were considered, this difference was found restricted to the first two zones, during the first period, and for the second and third zones during the second. This perhaps indicates that the death-rate exceeds the fresh production of active roots by wilted plants in the soil zones, which, according to season, are the main seats of their activity—a measure of reduction in vigour—under such conditions.

(*f*) *Yield of coton as influenced by wilt-incidence.*—Whether the differences in root vigour mentioned above, were reflected in yield differences of plants wilting during the bearing stage, was next studied. An attempt

was also made to find whether, in an infected soil, invisible attack on apparently healthy plants could be traced through a measure of their yield vigour. Plant yields of Kapas were recorded in 1933 and 1934, for apparently healthy plants, growing in wilt-infected and apparently uninfected field-patches. These were compared with yields of wilted plants, both dead and showing fresh growth. The average yields per plant are tabulated below with their statistical valuations.

TABLE IV.

Influence of "Wilt" on cotton yield.

Kapas, gms. per plant.

Healthy plants	Mean Yield	Comparison	n	t	p
(*a*) From unaffected patches	17·1	A. *Vs.* B.	47	1·7	>0·05
(*b*) From affected patches ..	12·0	A. *Vs.* C.	45	4·9	<0·01
		A. *Vs.* D.	40	4·5	<0·01
Wilted plants		B. *Vs.* C.	54	4·7	<0·01
(*c*) Dead	4·3	B. *Vs.* D.	49	4·3	<0·01
(*d*) Resumed fresh growth ..	4·2	C. *Vs.* D.	47	0·009	>0·05

These observations were taken from time to time over the harvest period on plants surviving to the bearing stage. The yield was lowered by wilting, whether the plant died or survived to grow later. The differences between yields of Kapas—both total and for each picking—of healthy plants from affected and unaffected patches were not significant. This shows that infection or no infection, healthy plants yield similarly.

(*g*) *Nutrition and Wilt-incidence Pot tests.*—The influence of soil differences in wilting pointed to another line of investigation.

Soil in pots (1933 and 1934) was treated with (I) inorganic nutrients in different proportions, (II) manures of widely different compositions and properties, and (III) dressings of substances calculated to effect a physical improvement of the soil.

The soil was inoculated with vigorous cultures of the fungus and sown with Malvi cotton. Repeated inoculations were made to ensure active infection throughout the period of observations. The identical soil in the pots of the 1933 experiments was given the same treatments in 1934.

Different degrees of virulence were observed in different treatments. The results are given in table V.

TABLE V.

Effect of Nutrients on incidence of wilt.

(*Pot culture*)

No.	Treatment	Mean percentage deaths		Increase in death in 1934—35 over 1933—34
		(1933—34)	(1934—35)	
1	N. (NH_4NO_3) Ratio ammonical N to Nitrate N 1 : 1 ..	50·0	100·0	+50·0
2	$N+P_2O_5$	50·0	91·7	+41·7
3	$K_2O+P_2O_5$ (1 : 1)	25·0	80·0	+55·0
4	CaO	16·7	65·2	+48·5
5	$K_2O+P_2O_5$ ($\frac{1}{2}$: 1)	8·3	41·6	+33·3
6	$K_2O+P_2O_5$ (1 : $\frac{1}{2}$)	8·3	33·3	+25·0
7	MgO	0·0	49·2	+49·2
8	Compost	0·0	29·2	+29·2
9	Glue	0·0	19·2	+19·2
10	Farm-yard manure ..	50·0	42·8	−7·2
11	Control	19·4	12·2	−7·2
	P	< 0·05	< 0·05	
	Sig. difference	27·2	30·8	

The salts used were ammonium nitrate for N, Potassium sulphate for K_2O, sodium phosphate for P_2O_5.

In series with added nutrients, the same relative differences were observed in nature though not in degree. In 1933, the highest virulence was

induced by nitrogen alone, and with phosphate ; it increased considerably in 1934. Potash with phosphate (1 : 1) in 1933 did not increase virulence significantly, but in 1934 this treatment reached the level, in this respect, of nitrogen alone and with phosphate.

When the ratio of potash and phosphate was altered in either direction, the increase of virulence in 1934 did not reach significance, though reduction in potash nearly did so.

Magnesia and lime did not differ from control in 1933 but gave a significant rise in virulence in 1934 of the same order as potash and phosphate ($\frac{1}{2}$: 1).

Compost and glue in both years showed no significant difference from farm-yard manure and control, though the first pair showed a numerical rise in virulence in 1934, and the second pair a fall.

In another series of pot cultures in 1934, the soil was mixed with heated soil (at the rate 60 cartloads per acre/two inches), ferrous sulphate (at the rate of 5 cwt. per acre/two inches) and slaked lime (at the rate of 1 maund per acre/two inches) in order to change its physical condition. Ammonium sulphate and Nicifos 22/18 grade (at the rate of 3 cwt. per acre/two inches) and farm compost and municipal compost (at the rate of 20 cartloads per acre/two inches) were mixed with each of these. Malvi cotton was sown after infection which was maintained as before. The results are shown in Table VI.

TABLE VI.

Physical treatments, manuring of soil and wilt-incidence in pot cultures.

Mean percentage death.

Manures	Physical treatments		
	Ferrous sulphate	Heated soil	Slaked lime
Nicifos 22/18	28·6	11·1	21·1
Ammonium sulphate	26·9	47·8	29·2
Farm compost	23·6	16·7	0·0
Municipal compost	6·7	25·0	8·3

	P	Sig. difference
Physical treatments	>0·05	..
Manures	<0·05	16·2
Interaction (physical treatments × manures) ..	>0·05	..

Ammonium sulphate	Nicifos 22/18	Farm compost	Municipal compost
.. .. 34·6	20·3	13·4	13·3

Nitrogenous treatments increased incidence as before in spite of texturel treatments. It is to be noted that the presence of phosphorus (Nicifos) had slightly modified the influence of nitrogen.

Field tests.—The uniform intensity of treatment in pot cultures is absent in the field, besides interaction of the subsoil plays its part. How far the results of the pot culture may be modified in the field was tested in 1934. A wilt-affected portion of a field was selected and the same physical and manurial treatments were given as in the pot cultures, and at the same rates. Malvi seed from healthy and diseased plants of the previous season, and healthy seed of Verum, was sown. The soil was infected as before. Results are given in Table VI A.B.C.D.

TABLE VI A.

Physical treatments, manuring of soil and wilt-incidence variety: Malvi (Healthy seed).

Physical treatments	Manures	Periods									P	Sig. diff.
		1	2	3	4	5	6	7	8	9		
Heated Soil	No manure	2·8	53·8 ↔	34·1 →	10·7	21·5 →	5·6	4·2	6·9	9·7	<0·05	11·7
,,	Nicifos 22/18	0·9	55·0 ↔	17·6	26·8	19·3 →	7·2	7·4	2·5	0	..	..
,,	Ammonium sulphate	0·6	62·4 ↔	27·5	22·4	26·8 →	0	11·1	3·6	13·8	..	..
,,	Municipal compost	0	52·4 ←	52·4 →	8·7	27·1 ↔	7·1	2·9	0	0	..	..
,,	Farm compost	0	50·0 ↔	23·6	12·4	16·5	10·5	3·8	3·3	4·2	..	..
Slaked lime	No manure	3·8	61·0 ↔	18·9	15·1	23·1 →	7·0	10·8	4·7	0·7	..	..
,,	Nicifos 22/18	0·6	36·0 ↔	19·0	12·7	17·1	10·9	2·8	0	2·9	..	..
,,	Ammonium sulphate	0	47·2 ←	36·7 →	9·3	29·5 ↔	0	2·5	8·9	3·6	..	..
,,	Municipal compost	1·3	45·2 ↔	16·4	10·0	39·7 ↔	0	16·7 ←	13·6	18·8	..	..
,,	Farm compost	0	43·4 ↔	28·3 →	11·3	28·1 ↔	7·1	4·2	8·5	0	..	..
Ferrous sulphate	No manure	1·7	53·6 ↔	28·6 →	5·5	18·6 →	0·5	13·8 ←	5·5	9·5	..	..
,,	Nicifos 22/18	0·8	46·0 ↔	22·2	13·6	33·9 ↔	8·3	2·3	0	4·0	..	..
,,	Ammonium sulphate	0	42·9 ↔	26·6	15·5	14·8	3·6	5·8	10·0	2·2	..	..
,,	Municipal compost	0	55·3 ←	45·3	38·3 →	8·3	0	13·3 ↔	0	0	..	..
,,	Farm compost	0	68·0 ←	28·7 →	3·6	13·6	4·6	6·0	0	0	..	..
Control	No manure	1·9	48·1 ↔	28·6 →	7·2	21·8 →	4·1	10·4	4·2	9·4	..	..
,,	Nicifos 22/18	0·6	47·3 ↔	16·6	14·1	25·4 →	6·3	6·6	0	4·8	..	..
,,	Ammonium sulphate	3·8	46·2 ←	33·2 →	14·0	13·9 →	1·8	9·5	14·3	3·1	..	..
,,	Municipal compost	4·4	45·5 ↔	24·6 →	8·9	26·5 ↔	6·4	9·4	1·8	0	..	..
,,	Farm compost	1·9	48·7 ↔	28·3 →	4·6	24·6 ←	16·0 →	2·6	3·6	4·2	..	..

TABLE VI B.

Physical treatments, manuring of soil and wilt-incidence variety: Malvi (Diseased seed).

Physical treatments	Manures	Periods									P	Sig. diff.
		1	2	3	4	5	6	7	8	9		
Heated Soil	No manure	3·1	63·4 ↔	43·5 →	10·4	1·7	0	5·6	16·7 →	0	<0·05	14·0
,,	Nicifos 22/18	2·5	56·0 ↔	26·7 →	3·1	60·0 ↔	0	0	4·2	0	..	..
,,	Ammonium Sulphate	0	54·0 ←	42·5	29·2 →	4·5	0	0	0	55·0 ←	..	..
,,	Municipal compost	1·8	60·5 ↔	27·7	13·3	15·6 →	0	6·3	0	21·4 ←	..	..
,,	Farm compost	0	52·0 ↔	25·0 →	5·0	0	5·6	0	16·7 ↔	0	..	..
Slaked lime	No manure	2·4	49·0 ←	23·8 →	3·8	7·5	4·1	15·7	7·6	10·2	..	..
,,	Nicifos 22/18	0	46·0 ↔	27·4	27·1	25·0 →	0	10·0	6·3	0	..	..
,,	Ammonium sulphate	0	33·5 ←	25·0	19·4	32·3	17·5 →	0	5·5	6·3	..	..
,,	Municipal compost	4·5	60·0 ↔	37·5 →	16·7	33·3 →	0	60·0 ↔	0	0	..	..
,,	Farm compost	15·4	54·8 ↔	20·2 →	5·6	25·9 ↔	0	28·3 ↔	10·0	0	..	..
Ferrous sulphate	No manure	2·4	52·0 ↔	23·9 →	4·3	20·4 ←	14·0	5·6	4·2	15·4	..	..
,,	Nicifos 22/18	0	54·8 ↔	22·7	11·6	10·0	8·3	0	0	0	..	..
,,	Ammonium sulphate	0	44·6 ←	44.1	29·5 →	0	0	5·0	5·6	0	..	..
,,	Municipal compost	0	61·3 ↔	15·8	12·5	48·7 ↔	5·6	18·8 →	0	0	..	..
,,	Farm compost	4·5	67·1 ↔	33·3 →	5·0	11·1	0	14·3 ←	16·7	0	..	..
Control	No manure	1·8	48·2 ↔	29·6	13·1	17·5	4·6	21·2 ↔	5·3	23·6 ←	..	..
,,	Nicifos 22/18	0	45·5 ←	15·3	9·7	21·9	8·6	11·3	5·0	9·1	..	..
,,	Ammonium sulphate	0	36·1 ←	41·0 →	7·7	12·9	6·1	29·4 ↔	5·0	5·0	..	..
,,	Municipal compost	0	52·1 ↔	27·6	19·0	26·4 →	2·8	0	6·3	0	..	..
,,	Farm compost	3·2	42·6 ←	26·1 →	7·5	19·3	10·3	3·6	9·5	9·0	..	..

TABLE VI C.

Physical treatments, manuring of soil and wilt-incidence. Variety Verum.

Physical treatments	Manure	Periods									P	Sig. diff.
		1	2	3	4	5	6	7	8	9		
Heated Soil	No manure	1·4	23·9 ↔	6·1	1·6	3·7	0·9	0·9	0·5	0·9	<0·05	9·0
,,	Nicifos 22/18	0	39·0 ↔	9·0 →	0	1·2	0	3·1	1·9	2·5	..	..
,,	Ammonium sulphate	0·8	29·6 ↔	6·9	0·9	5·8	0	3·4	0	1·0	..	..
,,	Municipal compost	0	28·8 ↔	9·0 →	0	1·4	0	0	0	0	..	..
,,	Farm compost	0	3·0	7·8	1·1	7·6	0	1·2	0	0	..	..
Slaked lime	No manure	0·6	42·7 ↔	18·1 →	4·9	12·6	4·3	1·5	4·3	3·2	..	..
,,	Nicifos 22/18	1·4	30·5 ↔	8·1	5·0	13·3	3·2	0	6·3	5·1	..	..
,,	Ammonium sulphate	0·8	36·3 ↔	20·3 →	3·5	8·9	2·2	0	2·3	0	..	..
,,	Municipal compost	2·3	38·3 ↔	20·5	12·3	14·7 →	0	0	0	6·7	..	..
,,	Farm compost	0·8	38·3 ↔	9·1	14·1	9·0	5·2	5·5	11·7	3·6	..	..
Ferrous sulphate	No manure	1·2	43·8 ↔	10·0	10·0	5·9	0·6	1·2	2·2	3·9	..	..
,,	Nicifos 22/18	0	34·0 ↔	11·2	3·5	1·9	1·7	0	0	3·6	..	..
,,	Ammonium sulpate	0·7	49·9 ↔	23·9 →	6·7	8·2	5·0	0	0	5·3	..	..
,,	Municipal compost	3·3	33·7 ↔	20·8 →	3·9	19·4 ←	0	0	0	5·6	..	..
,,	Farm compost	0	37·7 ↔	17·3 →	7·7	22·6 ↔	2·8	3·0	2·7	8·7	..	..
Control	No manure	0·8	30·6 ↔	8·8	3·1	8·1	1·7	4·2	3·1	3·5	..	..
,,	Nicifos 22/18	1·0	40·3 ↔	6·7	4·4	7·9	4·0	2·7	1·7	9·0	..	..
,,	Ammonium sulphate	0·3	31·8 ↔	20·0 →	7·4	15·0 →	1·7	0·7	1·4	12·1	...	..
,,	Municipal compost	2·7	30·5 ↔	17·5 →	0·7	12·2 ↔	1·2	0·8	3·1	4·3	..	..
,,	Farm compost	1·6	29·1 ↔	17·9 →	4·0	12·3 →	1·0	0	3·0	6·9	..	..

TABLE VI D. *Significance.*

	Verum		Malvi		Malvi diseased seed	
	P	Sig diff.	P	Sig. diff.	P	Sig. diff.
Periods	<0·05	2·95	<0·05	4·28	<0·05	6·50
Manrues	<0·05	1·07	<0·05	1·39	<0·05	1·73
Manures × physical treatments ..	<0·05	3·32	>0·05	..	<0·05	4·67
Manures × periods	<0·05	3·20	<0·05	4·2	<0·05	5·12
Manures × physical treatments × periods..	<0·05	8·7	<0·05	11·64	<0·05	13·96
Physical treatments..	>0·05	..	>0·05	..	>0·05	..
Physical treatments × periods	>0·05	..	>0·05	..	>0·05	..

For all varieties, irrespective of treatments, there was a significant rise in the incidence at the second, third and fifth periods. For Malvi plants, both from healthy and diseased seed, municipal compost showed a significantly higher incidence than Control and Nicifos. Ammonium sulphate was superior to Nicifos. With Verum however, ammonium sulphate and farm compost showed the highest incidence, there being no significant difference between farm and municipal compost. The physical treatments in general made no difference, but influenced the behaviour of manures with Verum and Malvi from diseased seed. For all varieties, however, manures influenced the periodic fluctuations and the physical dressing modified that influence.

It is thus clear that under field conditions application of the same manures, may give varying results, due to their interaction with the soil conditions and variety grown. Manurial experiments for wilt control have given inconclusive results in the past and will do so in future, unless such experiments are based (1) upon a definite knowledge of the interaction of soil and manure for the locality, and (2) upon equally definite information as to whether the interaction adequately satisfies the intrinsic requirements of the variety grown, estimated independently.

(*h*) *Composition of leaves of healthy and wilted plants.*—Mature leaves of the resistant Verum and susceptible Malvi plants—healthy and on the point of wilting—were collected and boiled in 85% alcohol. These were analysed.

The accuracy of this sampling was ascertained by the examination, for the presence of *Fusarium* of thirty plants about to wilt. Transverse

sections of (1) the tap-root four inches below the soil surface, (2) the hypocotyl and (3) the second internode of the stem, invariably showed fungus hyphæ in the vascular bundles. All samples except three, gave growths of fungus identified as *Fusarium* species, after incubation on cotton wool soaked in Richard's solution (Table VII A).

TABLE VII A.

Composition of mature leaves of healthy and wilted plants.
(Mean percentages on oven-dry basis).

Description	Varieties and conditions				Significance					
	Malvi		Verum		Varieties		Conditions		Varieties × conditions	
	Healthy	Wilted	Healthy	Wilted	P.	Sig. diff.	P.	Sig. diff.	P.	Sig. diff.
Organic matter* ..	85·04	87·04	86·26	87·30	>0·05	..	>0·05	..	>0·05	..
Ash	15·62	15·33	13·74	12·69	>0·05	..	>0·05	..	>0·05	..
Containing*										
Nitrogen ..	2·36	1·87	2·30	1·65	>0·05	..	<0·01	0·47	>0·05	..
Phosphate (P_2O_5)	0·70	0·55	0·93	0·80	<0·05	0·23	>0·05	..	>0·05	..
Potash (K_2O) ..	1·66	1·71	2·01	1·42	>0·05	..	>0·05	..	>0·05	..
Magnesia (MgO)	1·93	0·12	0·91	0·81	>0·05	..	>0·01	0·66	<0·01	0·93
Lime (CaO) ..	1·69	1·61	1·11	1·75	0·05	..	<0·05	..	>0·05	..
Ratios:										
Nitrogen/Phosphate	2·49	2·38	3·38	3·43	>0·05	..	>0·05	..	>0·05	..
Potash/Nitrogen	0·82	0·61	0·88	0·87	>0·05	..	>0·05	..	>0·05	..
Potash/Phosphate ..	3·17	2·07	2·29	1·76	>0·05	..	>0·05	..	>0·05	..
Magnesia/Phosphate	2·24	0·44	1·12	1·19	>0·05	..	>0·05	..	>0·05	..
Magnesia/Nitrogen	0·80	0·08	0·39	0·54	>0·05	..	>0·05	..	<0·05	0·71
Magnesia/Potash ..	1·06	0·12	0·45	0·65	>0·05	..	>0·05	..	<0·05	0·80
Lime/Phosphate ..	2·55	2·47	1·32	2·05	>0·05	..	>0·05	..	>0·05	..
Lime/Nitrogen ..	0·55	1·06	0·49	1·05	>0·05	..	>0·05	..	>0·05	..
Lime/Potash ..	0·81	1·64	0·62	1·15	>0·05	..	<0·05	0·63	>0·05	..
Magnesia/Lime ..	1·54	0·09	0·82	0·71	>0·05	..	<0·05	0·66	<0·05	0·93

TABLE VII B.

Composition of mature leaves of healthy and wilted plants.
(On oven-dry basis.)

Description	Varieties and conditions				Significance					
	Malvi		Verum		Verieties		Condition		Veriety and conditlon	
	Healthy	Wilted	Healthy	Wilted	P	Sig. diff.	P	Sig. diff.	P	Sig. diff.
Ratios:										
$K_2O/N+P_2O_5$ ..	0·64	0·46	0·62	0·57	>0·05	..	>0·05	..	>0·05	..
$MgO+/N+P_2O_5$ ..	0·61	0·09	0·28	0·36	>0·05	..	>0·05	..	<0·05	0·34
K_2O+MgO/N .	1·58	1·05	1·23	1·41	>0·05	..	>0·05	..	>0·05	..
K_2O+MgO/P_2O_5..	4·81	2·82	3·41	2·95	>0·05	..	>0·05	..	>0·05	..
$K_2O+MgO/N+P_2O_5$ ..	1·26	0·75	0·91	0·94	>0·05	..	<0·05	0·21	<0·05	0·29
$MgO+K_2O+CaO/N+P_2O_5$ ..	1·97	1·30	1·25	1·63	>0·05	..	>0·05	..	>0·05	0·66

There was no difference in the organic matter and ash contents of the leaves of healthy and wilted plants of both varieties. The nitrogen content of the leaves of wilted plants of both varieties was lower than that of the healthy ones. Phosphate content of Verum was higher than that of Malvi but health or wilting made no difference. The content of lime and potash showed no difference due to wilting. In Malvi the magnesia in wilted leaves was less than in healthy but not so with Verum.

Ratios of nitrogen to phosphate, and to potash, and of potash to phosphate did not differ for the healthy and wilted leaves of both the varieties. The ratios of magnesia to nitrogen, and to potash were lower for the leaves of wilted Malvi but not for wilted Verum. The ratio of magnesia to phosphate in the leaves of the wilted and healthy Malvi plants differed similarly but the values fell just short of significance (value of z obtained was 0·7433, while that required was 0·8163). Percentage of potash in proportion to lime is less for both varieties in wilted leaves than healthy ones while that of magnesia is so only for Malvi.

It is obvious that in the comparisions in Table VII B, the differences have become significant only through the influence of the ratio MgO/N.

It is clear that nitrogen deficiency and that of potash in proportion to lime is associated with the occurrence of wilt in general, and the susceptible Malvi shows a further deficiency of magnesia especially in proportion to

nitrogen and to potash and probably to phosphate. This point and the high percentage of phosphate associated with the resistant Verum, are suggestive, and may provide clues to the elucidation of the physiological nature of wilt resistance and may explain why resistant varieties or strains sometimes succumb. Perhaps varieties that experience a deficiency both of nitrogen and magnesia are "susceptible," while those that have the inherent capacity to collect sufficient magnesium in the same environment do not experience that deficiency, and are "resistant"; succumbing only where nitrogen supplies are unusually low. The deficiency of magnesia appears to be connected with the natural movement of replaceable magnesia in the soil profile. Perhaps the resistant varieties tackle their difficulties successfully through the adaptibility of their root-system, the nature of which adaptability is not yet clearly understood.

3. Conclusion and summary.

The above investigations have indicated that the incidence of wilt for both susceptible and resistant varieties may be influenced by several environmental conditions. Actual wilting of the plant is not due to insufficient soil moisture. Soil around the roots of wilted plants contained a higher percentage of bound water than that around the roots of healthy plants.

Root activity was less in wilted plants and their root death-rate always exceeded the fresh production of active roots in the upper 18 inches of the profile. This reduced activity was reflected in reduced yields. It was found that the incidence of wilt was affected by changes in nutrient supplied, but this influence was modified by soil conditions, and requirements of the variety, thus explaining the inconclusive results usually obtained in manurial trials to control wilt in the field. Though sometimes nitrogenous manures seem to increase wilt incidence, the nitrogen content of leaves of wilted plants was always found to be lower.

The apparent association of an abrupt rise in sunlight duration with increased wilt incidence, is perhaps connected with low nitrogen content. Sudden increase in photo-synthesis may lead to exhaustion of the vital proteins to a greater extent than their recuperation. Low nitrogen content of leaves of the wilt-affected plants may also disturb the balance of protoplasmic proteins, leading to partial disintegration, even in the absence of a change in the rate of photo-synthesis. How far the lowering of nitrogen in leaves, is a result of inability of the plant to absorb soil nitrogen or is due to its being intercepted by the fungus on its way to the leaf remains uncertain. At any rate, further work is needed to determine how and

to what extent, the fungus or the plant, or both are responsible for the observed modifying influence of environment on wilt-incidence.

It is clear however, that while investigating the possibility of wilt-control by soil management, the plant and the fungus can together be treated as one system.

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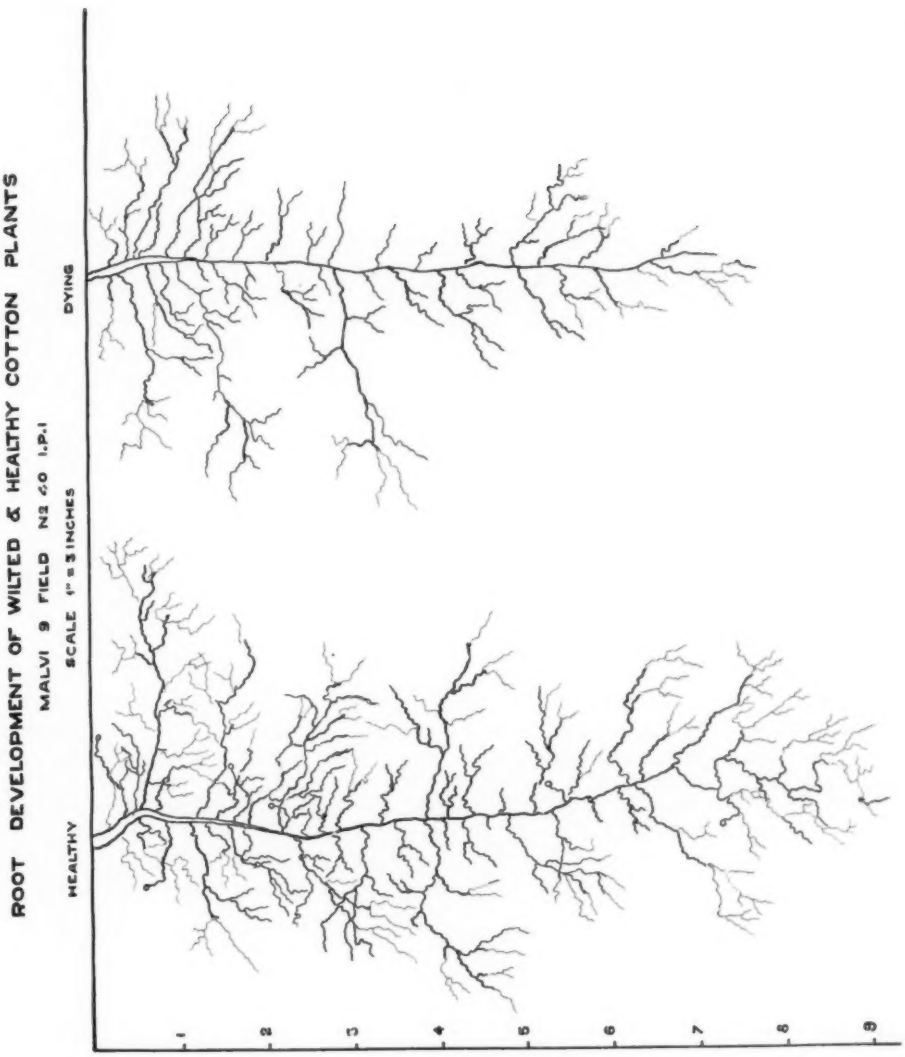
ROOT DEVELOPMENT OF WILTED & HEALTHY COTTON PLANTS
MALVI 9 FIELD N3 40 I.P.I
SCALE 1" = 3 INCHES
DYING
HEALTHY
1
2
3
4
5
6
7
8
9

A NOTE ON THE RELATION BETWEEN THE INTERNAL STEM STRUCTURE OF CERTAIN VARIETIES OF GRAM (*CICER ARIETINUM* L.) AND THEIR RESISTANCE TO CUTWORM ATTACK

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1. Introduction

A yield trial of two varieties of gram (*Cicer arietinum* L.), namely, Pusa Types 17 and 41, laid down in the Botanical Section in 1933-34, was vitiated because of a differential attack by the cutworm. The parallel strip form of lay-out had been adopted, and it was notable that whereas Type 17 was severely damaged in all the seven replications, Type 41 was attacked in a noticeably lesser degree. The reasons for this unequal attack were investigated, and the results obtained form the subject of the present paper. Not only was the amount of damage done, *i.e.*, number of branches cut per plant, greater in the case of the first-named variety, but a considerably larger number of plants was attacked. A count was taken of the number of plants injured, and the results are presented in Table I.

The name "cutworm" is commonly applied to the larvæ or caterpillars of several species of Noctuid moths which are more or less similar in appearance and habits. They generally feed, at night, on the tender and succulent shoots of low-growing vegetation, cutting the plants at the soil level. A single larva may cut off as many as 50 plants in a single night.

The most common species in Northern India are *Agrotis ypsilon* Rott., *A. flammatra* Schiff., *A. nigrum* Linn., *Euxoa spinifera* Hb., and *E. segetum* Schiff. Their larvæ feed on a wide range of plants including gram, peas, beans, tobacco, tomatoes, potatoes, sweet potatoes, cabbage, cauliflower, lucerne, etc.

TABLE I

Showing the Numbers of Plants of Types 17 *and* 41 *Damaged by Cutworms, in the Yield Trial Plots*

Plot No.	Variety	Number of plants attacked in each plot	Number of plants attacked, expressed as a percentage of the total number of plants in each plot
	Type		
1	17	107	68
2	41	66	41
3	41	74	43
4	17	110	67
5	17	96	58
6	41	62	38
7	41	85	52
8	17	154	95
9	17	129	83
10	41	101	61
11	41	73	45
12	17	111	67
13	17	127	77
14	41	68	42

Average percentage damage due to cutworm in Type 17 = 73·4
Average percentage damage due to cutworm in Type 41 = 46·0

2. *Material*

The eighty-four Pusa types of gram (Shaw and Khan, 1931) which were growing in the same field were examined for cutworm attack and classified into the following three classes : (A) Very slightly attacked ; (B) moderately attacked ; (C) severely attacked. As the plots of each variety were not replicated and the classification based on eye-judgment, this grouping must be considered to be rough, and perhaps somewhat arbitrary.

Four types were selected from each group, as being typical of the damage sustained, for detailed study. These types were :—

Group	A	B	C
Types ..	3, 26, 38, 79	9, 36, 41, 58	17, 46, 52, 82

In the following crop-year (1934–35), the grouping was checked and it was found necessary, in a few cases, to transfer types previously placed in the B group to the A or C groups, or *vice versa*. In no instance, however, did it become necessary to transfer a variety from the A group to the C group or *vice versa*. Of the twelve types selected for detailed study, the only change made was in the case of Type 9, which was transferred from the moderately attacked or B group to the severely attacked or C group.

3. Experimental Procedure and Results

Two possible reasons for the selective attack by the cutworm suggested themselves: (1) The comparative resistance of certain varieties might be associated with the physiology of the plant, particularly the possession of certain substances in the tissues of the plant, which rendered them attractive or unattractive to the insect or its larva. Some examples of such cases are given by Davidson and Henson (1929), Martin (1928), etc. (2) The resistance might be, mainly, merely physical, not due to any discrimination on the part of the insect, but to the toughness of the stems of certain varieties, which rendered them difficult or impossible to cut.

The first-mentioned possibility was tested indirectly by hand-picking the caterpillars at intervals and counting the number collected from plots of each variety. The results of two typical counts are given in the Table on the next page.

The figures just presented do not reveal any clear association between severity of attack and the number of cutworm larvæ found, and discountenance the possibility that any selection, in repect of varieties, was exercised by the moths (*i.e.*, in selecting places for laying eggs) or by the larvæ. Such selective action, if found, would indeed have been surprising on the part of a pest which attacks so many widely different plants. Attempts to associate resistance with place of collection of the variety, maturity, size of leaves and leaflets also proved unsuccessful. This line of investigation was therefore not persevered with and attention was concentrated on investigating the second possibility (*vide supra*).

A large number of sections were cut. As it was found difficult to obtain good microtomic sections, freehand sections were cut. For this purpose

Group	Variety	No. of caterpillars	
		Collected on 2—1—34	Collected on 6—1—35
A ..	3	23	0
	26	14	6
	38	24	11
	79	15	11
B ..	9*	13	2
	36	21	7
	41	13	4
	58	11	10
C ..	17	19	6
	46	23	11
	52	24	10
	82	15	5

* This variety was later transferred to Group C.

six plants of each of the twelve varieties were selected, care being taken that they were as typical as possible of the condition of growth of the whole culture. A small portion of the stem was then snipped off at a point about half an inch above soil level, *i.e.*, at the point at which the caterpillars usually cut the plants, and fixed in formalin-alcohol. This was done fifty-four days after sowing, at a time when cutworm incidence was at its maximum. The pieces of stem were then sectioned and stained with safranin and light green.

A study of the sections revealed the interesting fact that the internal stem-structure was probably the factor responsible for the differential preference shown by the cutworm. In the case of the severely attacked group, the stem diameter was comparatively small and the secondary wood weakly developed. In the case of the very slightly attacked group, on the other

hand, the stem diameter was large and the secondary wood very well-developed. The moderately attacked group was intermediate between these two groups in respect of stem diameter and resembled the very slightly attacked group in respect of development of woody tissues.

The internal structure of the stem, associated with the three groups, is illustrated in Plates XXIII, XXIV and XXV. The sections shown are of the 1933–34 material. Average measurements of the stem diameter for the same material are given in Table II.

TABLE II

Average Stem Diameters (mm.) of the Three Groups of Gram Grown at Pusa in 1933–34

Group A		Group B		Group C	
Variety	Average diameter	Variety	Average diameter	Variety	Average diameter
Type		Type		Type	
3	2·45	9*	1·58	17	1·58
26	2·76	36	2·20	46	1·58
38	2·82	41	1·83	52	1·46
79	2·79	58	1·92	82	1·52

* Later transferred to Group C.

It will be observed that both in its internal anatomy (Plate XXIV, Fig. 5) and in its average stem-diameter, Type 9 agrees with the members of Group C rather than with those of the group in which it was originally placed. As stated in Section 2, Type 9 was transferred to the C group on the basis of observations on the crop grown in 1934–35: this is in agreement with the results now presented, and the placing of Type 9 in Group B was obviously a case of incorrect diagnosis.

To verify the above results, sections of the same types grown at Pusa in 1934–35, were also cut. The internal stem-structure of these was found to be, in a relative sense, very similar to that described above. Type 9 was again found to resemble the varieties in Group C in regard to extent of development of the wood, but in stem-diameter it was more like the varieties

in Group B. Average stem-diameters for the 1934–35 material are giv n in Table III.

TABLE III

Average Stem Diameters (mm.) of the Three Groups of Gram Grown at Pusa in 1934–35

Group A		Group B		Group C	
Variety	Average diameter	Variety	Average diameter	Variety	Average diameter
Type		Type		Type	
3	3·44	9	2·38	17	1·94
26	2·65	36	2·52	46	2·05
38	2·73	41	2·24	52	1·75
79	3·16	58	2·25	82	1·92

In view of the fact that a rather small number of plants of each variety was studied (it was found impracticable to cut sections of large populations), no attempt was made to calculate standard errors and to determine the significance of the differences between the means presented in Tables II and III. Emphasis should therefore be placed on the trends and not on the absolute values.

The data which have been presented suggest that the severity of cutworm attack is directly associated with the internal structure and thickness of the stem, the caterpillars causing most damage to those varieties which typically possess slender, soft-wooded stems and hence offer less resistance to the insect's jaws. Such a conclusion is supported by observations which were made on varieties with thick, hard-wooded stems (*e.g.*, Type 79). Here the stem was sometimes seen to be partially gnawed near the base of the plant. Apparently the cutworm failed in its attempt to cut the stem near the base, and proceeded to the softer top portion and cut it off. Indeed in the very slightly attacked group, almost the whole of the damage was confined to weakly developed plants or to the extreme tips of the normally developed plants.

It should be emphasised, however, that stem diameter and the extent of development of woody tissues may be profoundly influenced by

nutritional and environmental factors. Under certain conditions, the stems of all or nearly all the varieties of gram become extremely woody, and the anatomical differences noted under a different set of conditions may be completely obliterated, and the relative positions of the varieties, in respect of the characters discussed, may even be interchanged. Thus, the same twelve varieties of gram grown under irrigation conditions at Karnal, in both the years, made much more vigorous growth than at Pusa. Sections cut from stems of plants of the same age (about eight weeks) as the Pusa lots, showed that the varieties were alike in possessing extensive development of secondary wood and large stem diameters. On the analogy of the results obtained at Pusa, it was to be expected that, by reason of their robust growth, the grams grown at Karnal would sustain very slight injury from cutworms. It is perhaps significant that such proved to be indeed the case.

As few cases of a simple, direct relation between the anatomical characters of a variety and liability to insect attack, such as has been described in this paper, appear to be known, it seems likely that further investigations of this nature, with other varieties of gram and with other crop plants, may yield data of both interest and value. The demonstration that such a relation exists, in the case of the gram varieties studied at Pusa, suggests as an obvious measure for the control of the cutworm pest, the selection of tough-stemmed varieties for cultivation in tracts known to be particularly prone to the pest.

I am indebted to Dr. F. J. F. Shaw, the Imperial Economic Botanist and Director, for the suggestion to take up this investigation. My thanks are also due to Dr. H. S. Pruthi, the Imperial Entomologist, for information relating to North Indian cutworms and their habits, and to Messrs. Hukam Singh, Pushkar Nath and Mohammad Umar, for assistance in the field and laboratory work.

The photomicrographs were kindly taken by Dr. M. Mitra in the Mycological Section, by permission of the Imperial Mycologist.

4. Summary

1. Much more severe cutworm damage was noticed in Pusa gram Type 17 than in Type 41. The two varieties were grown in paired plots in a yield trial conducted at Pusa in 1933–34. The reasons for this differential attack were investigated.

2. Caterpillar counts in all the 84 Pusa types of gram showed no obvious relation between severity of attack and the number of caterpillars found. This suggested that probably no preference in respect of varieties was exercised either by the moths or the larvæ but that some physical attribute of

the stem was responsible for the unequal damage in varieties. To test this possibility, cross-sections of the stems of very slightly attacked, moderately attacked and severely attacked varieties were studied.

3. It was found that very slightly attacked varieties were characterised by a large stem diameter and extensive development of woody tissues, whilst severely attacked varieties had a comparatively smaller stem-diameter and the secondary wood was weakly developed. Moderately attacked varieties were intermediate between the two groups in respect of stem-diameter, and like the very slightly attacked group in respect of extent of development of woody tissues.

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EXPLANATION OF PLATES

Plate XXIII

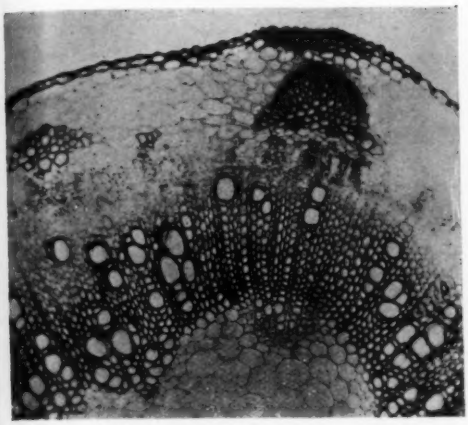
Fig. 1.—T.S. stem of gram Type 3. × 80.
Fig. 2.—T.S. stem of gram Type 26. × 80.
Fig. 3.—T.S. stem of gram Type 38. × 80.
Fig. 4.—T.S. stem of gram Type 79. × 80.

Plate XXIV

Fig. 5.—T.S. stem of gram Type 9. × 80.
Fig. 6.—T.S. stem of gram Type 36. × 80.
Fig. 7.—T.S. stem of gram Type 41. × 80.
Fig. 8.—T.S. stem of gram Type 58. × 80.

Plate XXV

Fig. 9.—T.S. stem of gram Type 17. × 80.
Fig. 10.—T.S. stem of gram Type 46. × 80.
Fig. 11.—T.S. stem of gram Type 52. × 80.
Fig. 12.—T.S. stem of gram Type 82. × 80.



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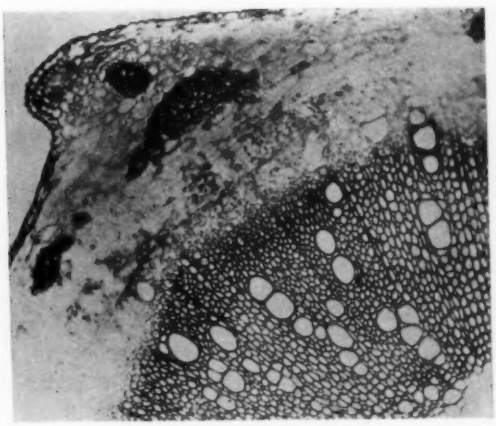

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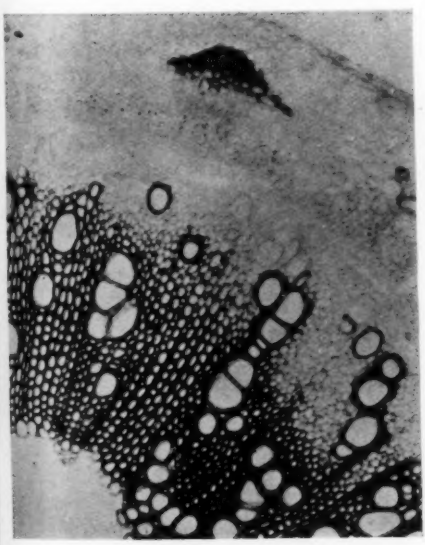

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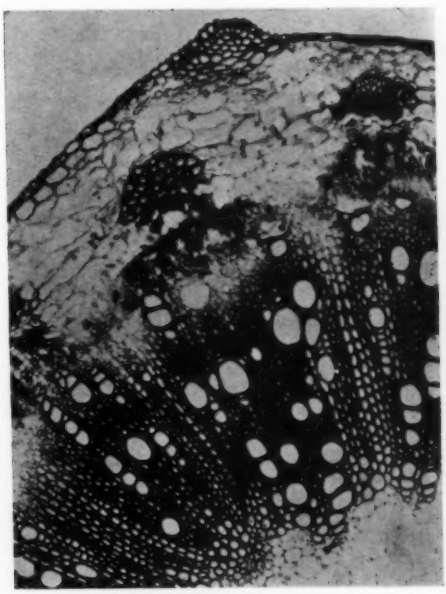

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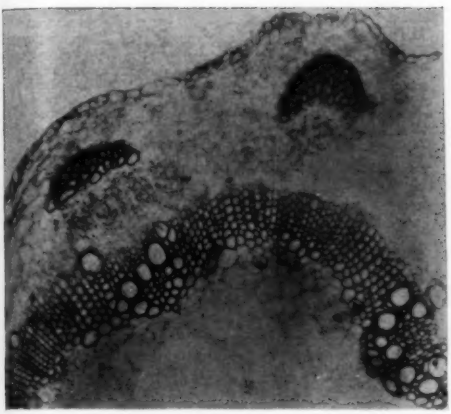

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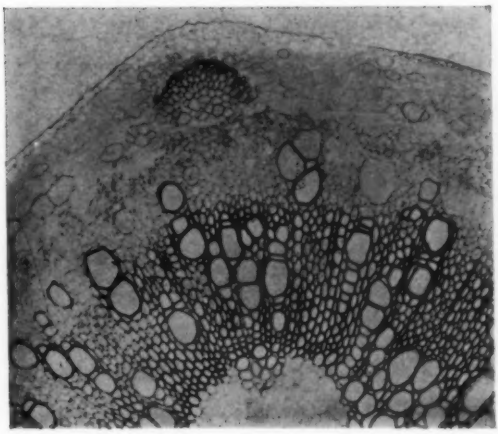

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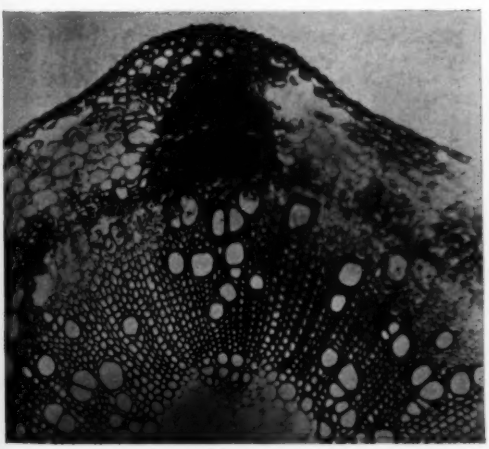

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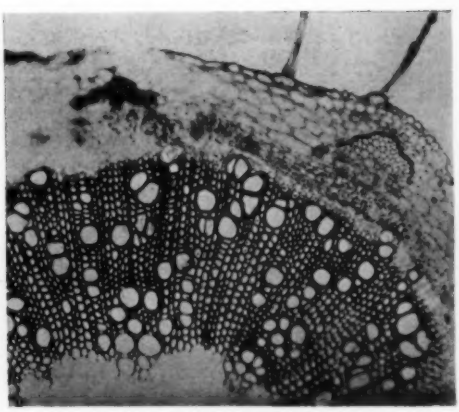

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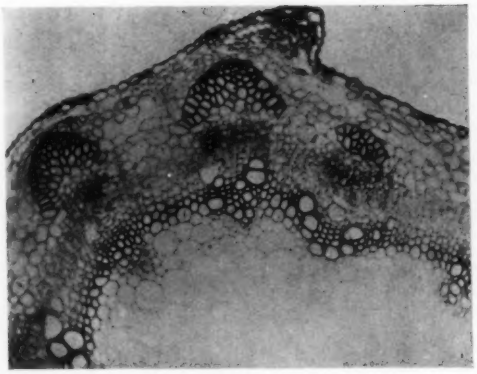
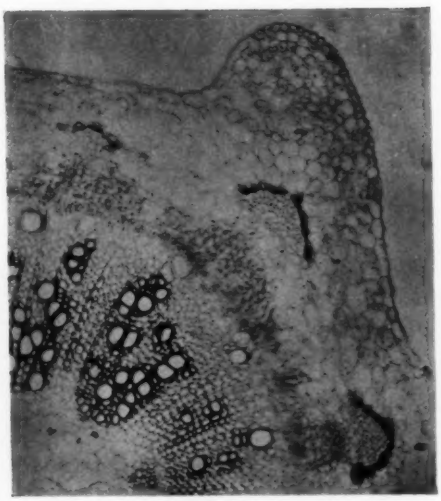


FIG. 9.

FIG. 10.



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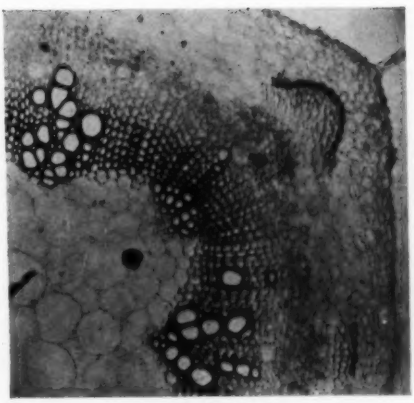

FIG. 11.

STUDIES IN DISEASE RESISTANCE—II. LEAF-ROLL AND RED LEAF OF AMERICAN COTTONS.

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I. Introduction.

(1) *The Disease.*—American cottons never made a satisfactory growth on black cotton soil at the Institute. The leaves rolled downwards, the lustre was reduced and brittleness followed (Plate XXVI). Frequently red colour developed on the fringes of leaves, or as patches on intervascular portions, or both. Leaves sometimes became wholly red. The red colour also appeared on other green parts of the plant. The affected leaves started drying from the edges and ultimatley shed. The disorder appeared at all stages of growth. Growth stopped if the attack was sufficiently intense and in young plants very often led to their death. When affected early, the crop gave a low yield of poor quality or even no yield.

Recovery was possible but the disorder often appeared again and again after an interval of recovery. When usually in a moderately affected crop healthy and diseased plants stood side by side.

The Cambodia crop in tank areas at Badnawar in Dhar State and in Jaipur was generally free from this disease, though not entirely so in two recent seasons of heavy rainfall. In Jodhpur the disease has not yet been reported. Other American varieties, however, invariably developed the disease everywhere in Malwa and Rajputana.

In the arid region of Sriganganagar in Bikaner State, the canal-irrigated Punjab Americans are usually affected in the later period (October), though it is not uncommon to find affected plants earlier (July).

Development of red colour and frequently rolling of leaves have been observed at the Institute on *desi* cotton as well as other crop plants and weeds, simultaneously with its occurrence on the Cambodia crop.

Red colour also appears on leaves without any preliminary rolling; then the unaffected green portion often turns yellow.

FIG.H. UNAFFECTED CONTROL IN THE OPEN.

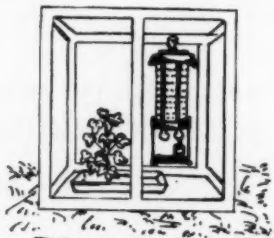

FIG.2A. HUMID CHAMBER.

FIG.B. PROTECTION BY SHADE.

FIG.C. UNSHADED CONTROL.

FIG.D. LEAF ROLL BY AMMONIA. FIG.E. LEAF ROLL BY ALKALI. FIG. G. LEAF ROLL BY RAIN

FIG. F. LEAF ROLL BY ALKALI UNDER SHADE.

FIG. I. LEAF ROLL BY MECHANICAL DEFLOCCULATION

FIG. 2.

(2) *Its occurrence in India and outside.*—In India, the disease has been observed on American cottons in the United Provinces (Burt and Haider, 1919), Dharwar (Kottur, 1920), Khandesh (Prayag, 1927–28), Punjab (*Ann. Report*, Montgomery Agric. Sta., 1929–30), Hyderabad, Deccan (Sawhney, 1932), and Sind (*Ann. Report*, Indian Central Cotton Committee, 1933–34) and elsewhere. It has also been recorded in other places outside India ; New Guinea (Evans, 1926), Nigeria (Jones and Mason, 1926 ; Brown and Casement, 1932–33), Nyasaland (Sampson, 1927) and Uganda (*Ann. Rept.*, Dept. of Agric., 1931). Jones and Mason considered it to be a physiological disorder.

The disease has been variously ascribed to humid conditions, aphis and jassids, water-logging, and wide spacing. The protective influence of shading and close spacing, and recovery during dry weather have also been mentioned.

II. Experimental Results.

Pot-cultures were grown to verify the above suggested causative factors, the Cambodia variety (a pure strain–Indore 1–for quantitative observations) was used in all experiments unless otherwise mentioned. Black cotton soil from the surface of fields was always used. Each treatment had four replications.

(1) *Humid conditions.*—It was not clear whether by "humid conditions", the wetness of soil, air, or both was intended. At the Institute, the disease was observed to develop both during wet spells with saturated soil and high atmospheric humidity as well as during the dry weather following rains. Its appearance at Sriganganagar in the later stages of the crop was surely not associated with wet conditions either of soil or air. At Indore, it was usual to secure by irrigation a recovery in the Cambodia crop when affected during dry weather in October.

Leaf-roll did not appear even after 15 days with atmospheric humidities varying from 75% to 85% in a preliminary experiment conducted in August 1929 in covered pots made impervious to water and with the soil always maintained in a saturated condition. This experiment was repeated in the same season in pots sunk in soil of a cropped field. Plants were simultaneously subjected to the following four treatments :

(*a*) wet soil and wet air ;
(*b*) dry soil (with moisture sufficient for plant growth), and wet air ;
(*c*) dry soil (as above) and dry air ;
(*d*) wet soil and dry air.

Soil round the plots belonging to treatments (*a*) and (*d*) (wet soil) was also kept freely watered. Humidity of the air was controlled by covering the pot with a ventilated glass chamber (Fig. 2 A) with a wooden frame with basins of water or calcium chloride—trays placed in the path of the in-coming air.

Experiments under treatment (*a*) lasted from 20th September 1929 for a month and treatments (*b*), (*c*) and (*d*) till 14th October. The humidities in each case were as follows :

TABLE I.

Humidities around the Plants in Pot-Cultures.

Treatment	Relative humidity (%)	
	Inside	Outside
(*a*) Wet soil and wet air	93 to 100	52 to 96
(*b*) Dry soil and wet air	62 to 96	56 to 79
(*c*) Dry soil and dry air	50 to 70	52 to 76
(*d*) Wet soil and dry air	67 to 77	71 to 79

All the plants remained unaffected except those in treatment (*a*) which showed yellowing and rolling of leaves at the end of the experiment but quite insignificant in magnitude compared with that observed in nature.

(2) *Shade.*—During the wet part of the season supposed to favour the disease, plants were shaded from sunlight between 10 a.m. and 4 p.m. Incidentally and unavoidably they were protected from rain also. The shaded plants turned dark green, grew to twice the size of the controls (Fig. 2 B and C) with no signs of the disease. This experiment was continued during the dry period also with the same result. Both the shaded and unshaded plants developed no marked rolling of leaves. The experiment was thus inconclusive.

(3) *Water-logging* : (Fig. 3).—Continuous water-logging of the soil with oxygen-free water supplied from a reservoir through the bottom of impervious pots for 16 days did not result in the development of the disease.

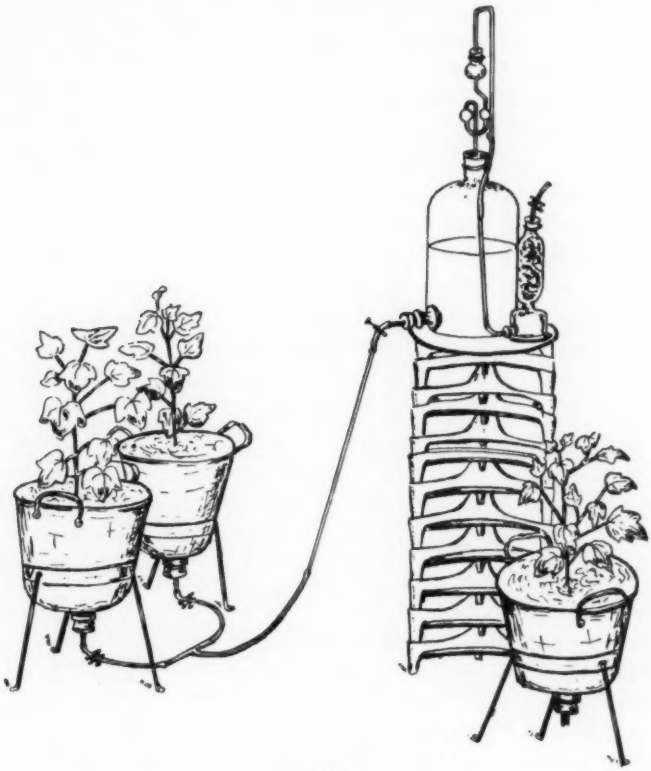

FIG. 3.

(4) *Soil deflocculation*: (*a*) Deflocculation of soil by repeated doses of dilute ammonia in pots in the open between September 9th and October 14th produced appreciable rolling immediately after the treatment (Fig. 2 D). The plants, however, very soon recovered and grew more vigorously, due obviously to increased nitrification. The rolling never reached that degree which is found in the field.

(*b*) Dilute sodium carbonate solution was then used in repeated doses between 19th September and 20th October 1929 on another set of pots kept in the open. More pronounced roll was produced in this case but it was still less than in field conditions (Fig. 2 B). There was also a tendency to recover soon after the treatment.

(*c*) The same treatment was then given to half the plants under shade (Fig. 2 F). Rolling was produced between October 7th and October 20th

during warm weather in spite of shade and was also followed by red leaf, controls remaining unaffected. In addition to the supposedly protective effect of shade, the only apparent difference between the pots under shade treated with sodiumcarbonate and the corresponding ones in the open, seemed to be that the surface soil of those under shade was never allowed to dry out.

(*d*) *Mechanical deflocculation of soil after long-continued wetting.*—Immediately after the heavy rains in August, leaf-roll appeared in a set of control plants in field pots that happened to be in the path of the surface run-off. Red leaf also followed (Fig. 2 G). Some of the plants remained permanently stunted while others recovered. Other control plants not subject to run-off remained unaffected (Fig. 2 H). It appeared that the mechanical effect of a brisk run-off was also a factor. The soil in pots with stunted plants dried to very hard clods. The degree of decrease of disease appeared to follow the friability of the soil.

At the same time, it was noticed that Malvi cotton in Field 1, bullock-hoed to form 3 inch-ridges across the slope remained unaffected though it was water-logged upto the point of leaf-fall. Portions of the unridged Malvi crop in Field 6 which suffered only from surface-wash showed slight leaf-roll and more of red leaf. This effect of surface-wash was extensively observed on the Malvi crop later in 1932.

It seemed from these observations that the disease appeared when the wet soil was deflocculated by chemicals or by the mechanical force of surface-wash or beating rain. The latter point was verified by subjecting soil in pots carrying vigorous healthy plants to prolonged saturation and then hand-kneading the surface inch, in the dry weather of October and under the protection of shade—both conditions supposed to be unfavourable for the disease. Leaf-roll appeared on the second day and developed to field intensity, red leaf following (Fig. 2 I). Controls water-logged, but unkneaded and protected by shade, did not develop the disease, nor without water-logging did it appear by hand-kneading even when not protected by shade. The disease, found under natural conditions (at any rate on black soil), appears to be mainly due to mechanical kneading of soils after prolonged saturation. The necessity of previous saturation probably indicates that the mechanical effect can only operate after the soil undergoes a change in its properties.

(5) *Sudden fall in temperature.*—Incidence of disease is sometimes attributed to this cause (Howard, 1921). Temperatures of falling rain-water were recorded along with those of the air immediately before it fell. Soil temperatures, being known to run at a lower range parallel to atmospheric ranges (McKenzie Taylor, 1927), were not determined. Some of the readings are given below :—

TABLE II.

Temperature Fall Due to Heavy Rain.

Date	Time	Temp. of air immediately before rain °F.	Temp. of rain °F.	Quantity of rain Inches	Temperature of air, previous day °F.	
					Max.	Min.
July 12th	7 a.m.	78	73	2·78	84	77
July 19th	,,	75	72	1·58	80	75
Aug. 26th	,,	71	68	3·61	76	70

Chilling due to rain is not likely to be a factor as it does not exceed the daily range in temperature.

It is, however, possible that the plants may be affected if their root-activity is reduced through the temperature falling below the optimum range, suddenly or slowly. Here, the following observations seem to be interesting.

In 1932, it was observed that during monsoon rain the temperature of surface soil (at 6 ins.) was higher than that of the sub-soil (at 15 ins.), but the position was reversed soon after the rain had stopped, the surface readings being 2°–3° F. lower than those of the sub-soil. This persisted for several days, as long as the surface was sufficiently moist, and was obviously due to cooling by evaporation. Such a cooling of the soil-zones with the range of root-activity may occur during breaks in the rainy season as well as in the beginning of the dry season in October after the cessation of rains until the upper zone becomes dry. If, as will be shown later, reduced root-activity is connected with the appearance of the disease, lower temperature may thus favour the disease. This seems to be corroborated by the common observation that the disease is more prevalent in the cold weather than in summer. Apearance of the disease even at higher temperatures, however, indicated the existence of other more powerful factors which were then studied in preference.

The above experiments made it quite clear that the factors which had been suggested possibly had little direct connection with leaf-roll. The disease could invariably be produced by the deflocculation of even an inch of surface soil either by chemical or mechanical means which also, when sufficiently intense, could produce the disorder in *desi* varieties usually considered immune.

(6) *Insects.*—Aphis (Burt and Haider, 1919) and Jassids (Sawhney, 1932) have been found associated with red leaf on cotton. It is not clear,

however, why aphis or jassids should leave some plants unattacked even if they grow side by side unless predisposing factor is assumed as mentioned by Metzger *et al.* (1935) for Japanese beetle. A possible difference in the sap composition was therefore sought for.

(7) *Reaction of the leaf-sap.*—pH values of the water-extracted sap were determined for three years (1931–34) on healthy and diseased mature leaves as well as green and red portions of the diseased leaf of Cambodia. The values were significantly higher for the sap of healthy green leaves than those of both the green and red portions of the diseased leaf. There was no difference between the sap of green and red portions of the same leaf.

TABLE III.

pH Values of the Sap of Healthy and Diseased Leaves of Cambodia.

Comparison between	Mean pH		*n*	*t*	*p*
	Healthy	Diseased			
1. Healthy green (whole leaves) and rolled green portions of leaves ..	5·82	5·69	12	2·43	<0·05
2. Healthy green (whole leaves) and red portions of leaves ..	5·82	5·61	12	3·53	<0·01
3. Green and red portions of rolled leaves ..	5·69	5·61	14	2·06	>0·05

(8) *Osmotic pressures of leaf-sap.*—The diseased conditions, therefore, seemed to be associated with a change in chemical composition of the leaf-sap. This was also indicated when osmotic pressures of the sap of healthy and unhealthy mature leaves were determined by the plasmolytic method of De Vries (De Vries, 1884). Irresepctive of season and locality, osmotic pressure of the sap of healthy leaves was higher than that of unhealthy leaves which therefore seem to contain less soluble matter.

TABLE IV.

Mean Osmotic Pressures (in Atmospheres) of the Leaf-Sap of Healthy and Diseased Cambodia Plants (Leaf-Roll).

(Wad and Mogre, 1934).

Healthy	Diseased	*n*	*t*	*p*
6·7	5·2	120	6·87	<0·01

(9) *Composition of leaves.*—Samples of healthy and diseased mature leaves were collected at Indore and Jaipur (in 1932–34) and immediately boiled and preserved in 90% alcohol, until analysed. Some of the results are given below :—

TABLE V.

Composition of Healthy and Diseased Mature Leaves of Cambodia (on oven-dry basis.)

Description	Mean %		P	Significant difference
	Healthy	Diseased		
*Organic matter ..	86·24	86·85	>0·05	..
Ash ..	13·75	13·14	,,	..
*Containing :—				
Nitrogen ..	1·71	1·21	<0·05	0·39
Phosphate (P^2O^5) ..	0·83	0·76	>0·05	..
Potash (K_2O) ..	2·00	1·63	,,	..
Magnesia (MgO) ..	1·8	1·39	,,	..
Lime (CaO) ..	1·21	1·21	,,	..
Ratios :—				
Nitrogen/phosphate ..	2·23	1·83	,,	..
Potash/nitrogen ..	1·14	1·56	,,	..
Potash/phosphate ..	2·40	2·34	,,	..
Magnesia/nitrogen	1·11	1·32	,,	..
Magnesia/phosphate	2·42	1·65	,,	..
Magnesia/potash ..	1·23	0·92	,,	..

The organic matter and ash showed no difference but nitrogen was significantly lower in the diseased leaves. Though statistically not significant, the percentages of phosphate, potash and magnesia were lower in

TABLE V (*a*).

Composition of Healthy and Diseased Mature Leaves of Cambodia (on oven dry basis.)

Description	Mean ratio		P	Sig. Diff.
	Healthy	Diseased		
Ratios :—				
$K_2O/N + P_2O_5$ ..	0·76	0·89	>0·05	..
$MgO/N + P_2O_5$ ..	0·74	0·68	>0·05	..
$K_2O + MgO/N$ ..	2·26	2·88	>0·05	..
$K_2O + MgO/P_2O_5$ ..	4·83	3·99	<0·05	0·73
$\frac{K_2O + MgO}{N + P_2O_5}$ ..	1·51	1·57	<0·05	..
$K_2O + MgO + CaO/N + P_2O_5$	1·99	2·25	>0·05	..

diseased leaves. The lime-content did not differ. The ratios of potash to nitrogen and phosphate and that of magnesia to nitrogen did not differ significantly. The ratios of magnesia to phosphate and to potash and of nitrogen to phosphate though not statistically significant were numerically lower. Magnesia and potash taken together were deficient with respect to phosphate. Acid-base balance (total) did not differ [Table V(*a*)].

The consistently lower numerical values for magnesia and some of its ratios appear to need further investigation. Perhaps the appearance of red leaf alone and in association with the rolling of leaves will have to be separately examined. It is suspected that the natural movement of replaceable magnesium in soils may play a part in the development of at least one of the phases of the disease.

The chemical differences in the composition of healthy and diseased leaves suggested a possibility of differences in the composition of the medium of growth-soil-water—which were therefore sought.

(10) *Reaction of soil solution.*—Determinations were made of the reaction of soil from different horizons within the root-zone of pairs of healthy and unhealthy Cambodia plants growing side by side. The pH values varied from 7·73 to 8·89, were indiscriminately distributed and showed no

difference between 'healthy' and 'diseased' soils. The concentration of the soil solutions was therefore investigated.

(11) Osmotic pressures of soil-water around the roots of healthy and diseased plants were determined for 0–3 ins. and 12–15 ins. depths by the freezing point method of Bouyoucos and McCool (1915) (Table VI).

TABLE VI.

Mean Osmotic Pressures (in Atmospheres) of Soil-Water from Zones around Healthy and Diseased Cambodia Plants.

(Wad and Mogre, 1934).

Description	Healthy	Diseased	*t*
1. Upper zone 0–3″ ..	3·59	13·37	4·94
2. Lower zone 12″–15″ ..	10·37	17·22	3·43
3. Percentage drop from lower to upper zone	62·86	22·31	3·84

$n = 10$; $P < 0{\cdot}01$ for all.

Osmotic pressures of soil-water were less around roots of healthy plants than diseased ones, at any rate down to a depth of 15 ins., but the percentage fall in osmotic pressures from lower to upper layers was greater around roots of healthy plants than diseased ones. This is taken to indicate accumulation of solutes due to poorer mobility in the soil around the roots of diseased plants. Ruizhenkova (1932) found that the cotton plant was profoundly influenced by salt concentration in the upper 50 cm. soil layer.

Differences in concentration of soil salts must obviously influence the plants through their roots. An examination of the root-systems of healthy and diseased plants was then begun.

(12) *Root-activity of healthy and diseased plants.*—Adjacent pairs of Cambodia plants, healthy and diseased, were chosen. The technique followed in this study was the same as that described elsewhere (Ambegaokar and Wad, 1935).

The total root-length of healthy and diseased plants at each depth showed no difference. The ratio of total roots in the upper foot to those below also did not differ. The active root-length of diseased plants below one foot depth was significantly less and the length of dead roots was significantly greater in the upper foot for diseased plants than healthy. Active root-length in the upper foot and the dead root-length below one foot were

TABLE VII.

Differences in Root-Activity of Healthy and Diseased Cambodia Plants.

	Soil depth	Mean root length in inches		t	P
		Healthy	Diseased		
1. Total roots	0–12″	159·1	108·8	1·96	>0·05 <0·1
2. ,, ...	12″ downwards	106·6	52·1	1·84	>0·05 <0·1
3. Active roots	0–12″	11·0	5·6	1·43	>0·05
4. ,, ...	12″ downwards	40·5	6·1	3·67	<0·01
5. Dead roots	0–12″	4·8	15·7	3·18	<0·01
6. ,, ...	12″ downwards	7·6	14·6	1·25	>0·05
7. Percentage of total roots below 12″ to those within the first foot..	—	74·3	40·3	1·65	>0·05
8. Active/Dead	0–12″	2·29	0·36	—	
9. ,, ...	12″ downwards	5·33	0·42	—	

(n = 20 ; same for all.)

alike in healthy and diseased plants. It appears that the extensive root-death in the upper zone reduces the activity of the roots in the lower zone in diseased plants (Plate XXVII). Similar reduction in root-sytem has been noted by Nutman in his studies on 'die-back' of coffee plants (Nutman, 1933).

(13) *Influence of the disease on yields.*—Practically no yields are obtained when the disease appears in early stages but when it appears later during boll-formation as at Sriganganagar in 1934, yields are unaffected, as is indicated in the table.

Total yield of kapas was not affected. Most of the yield of diseased plants was obtained in the first picking, falling in the proportion of 4 : 2 : 1 at each picking while, the yields of the second and third picking of healthy plants were nearly five times that of the first picking. The first picking of diseased plants and the second of healthy ones yielded equally. This shows that the diseased crop could not mature properly normally—confirmed by the observed partial bursting of bolls. This may be the cause of reduced quality in a diseased crop with normal yield. A Cambodia crop affected by red leaf at Dhar, Malwa, during 1934-35 gave a lint length of about 19 millimeters only.

TABLE VIII.

Yield Differences in Healthy and Diseased Plants, 1934–35.
Variety: P. 289F at Sriganganagar, Bikaner State, Rajputana.
Mean yields of seed cotton in gammes per plant from 30 random pairs.

Condition	First picking	Second picking	Third picking
Healthy ..	77·67	364·0	323·33
Diseased ..	411·33	209·67	97·67

	P.	Sig. difference
Health and disease	> 0·05	
Pickings	< 0·05	5·22
Interaction :		
Health, disease × pickings	< 0·01	6·63

III. Discussion and Conclusion.

Cultivation of American cottons in India and similar regions is greatly handicapped by this disease. The pot-culture results show that it may develop under different atmospheric environments, provided the soil surface becomes puddled after prolonged saturation. In monsoon areas this happens naturally by rains at a time depending on their distribution and intensity during the season, and in arid regions, when the crop is continuously irrigated. In the sandy open soils of Jaipur or the manured opium fields on the black cotton soils of Malwa, this condition develops when the rains are exceptionally heavy.

On ordinary black soils, the disease has been prevented by applying to the soil profile, farmyard manure or compost, or by a dressing of heated soil mixed to a depth of six-inches (Wad, Mogre and Bhargave, 1934). Accumulation of salts in the upper soil zones around diseased plants indicates decreased permeability. Addition of organic matter or dehydrating the soil colloids by heat seems to retard this decrease.

Without previous long-continued saturation, puddling has little effect on black soil, but saturation even of sandy soils produces the unfavourable condition. It appears that the bacterial activity and probably a change in the replaceable base status, associated with prolonged saturation of soils, bring about an alteration in the clay complex in the absence of which the environment does not become unfavourable. This change seems to be

occurring normally in all soils during the crop-season, differing only in degree, and the influence of this deterioration on plant growth seems to depend on whether the plants can produce sufficient fresh roots to compensate for the root-death-rate. Even in normal cotton crops, it has been found that the death-rate increases with the advance of season, as shown in the table.

TABLE IX.

Mean Percentage Deaths of Cotton Roots at Different Stages of Growth.
(Apte, unpub.)

Sowing dates : (1) Pre-rain-sown, June 10th, 1933.
(2) Rain-sown, June 27th, 1933.

Varieties	Stages of growth		
	One month after sowing	Two months after sowing	At second picking
Malvi ..	7·4	53·2	45·7
Cambodia ..	4·5	34·4	45·2

Interaction :	P.	Sig. difference
Variety × states of growth	< 0·05	7·7

The results of the root-studies in the present investigation show that the diseased plants always have a greater root-death-rate than the fresh production of active roots. This upsets the balance of growth and lowers the plant's vigour as is seen by lower sap concentration and lower nitrogen content of the diseased leaves. An unsuccessful struggle to produce fresh root-growth to compensate the higher root-death-rate of diseased plants in the upper zone is indicated by the equality of active root-length for both healthy and diseased plants in that zone. This struggle is presumably an attempt to maintain root-activity especially in the lower zones by increased absorption of plant food, from the upper zone, mainly nitrates, which are low late in the season and in humid conditons (Wad and Aurangabadker, 1935). In pot-cultures soils treated with nitrogen, either alone or in combination, grew Cambodia plants free from disease. That the trouble is mainly due to inadequate nutrition resulting in weakened growth-activities is also indicated by the absence of yield from early-affected plants and the opening of immature bolls when affected during the mature stage.

Further work is needed before the exact rôle can be described which is played by the changed soil environment in reducing the root-activity. Several possibilities suggest themselves : (i) that lowered root respiration reduces root vigour, resulting in unbalanced absorption and assimilation of nutrients, (ii) that the concentration of the available nutrients is too low to produce or maintain a root-system large enough to collect all the plant needs, (iii) that the ratio of nutrients that could be absorbed is unsuitable to maintain the normal balance of metabolic activities.

Even though the complete picture cannot yet be drawn it is clear that a practical solution lies in increasing the resistance of the soil to long-continued saturation with its deleterious effects, which resistance must be maintained sufficiently strong till the end of the crop season. Initial fertility alone does not provide enough resistance, *e.g.*, Rajputana soils can replace the nitrogen absorbed by plants much more rapidly in the initial stages than the unmanured black cotton soils but their nitrifying activity is reduced much faster in the later stages (Wad and Aurangabadker, 1935). The disease appears early on black cotton soils and later in Rajputana. This decrease in rate of nitrification is retarded by organic matter. Manured garden lands on black cotton soil seldom show the disease. Thus the line of fruitful research in the future appears to be to find out for each locality a suitable method of soil fertilizing (in the widest sense of the term) so that its resistance is maintained up to the end of the season. In regions like Malwa where the fertility level is initially low, it must also be increased.

IV. Summary.

Leaf-roll and red leaf of American cottons has been described. Results of pot-culture experiments indicate that it may develop under different atmospheric environments, provided the soil surface becomes puddled after prolonged saturation.

These bad soil conditions appear during the monsoon period in Malwa but later in Rajputana. Accumulation of salts found in the upper layers of soil around the diseased plants, shows the poor permeability of these layers.

Death-rate of roots of diseased plants in the upper layer of soil was found to be too high to be compensated by the rate of active-root production. The total active root-length of diseased plants in the lower zone was less than that of the healthy plants. Whether this reduced root-activity of diseased plants is due to the lowering of respiration or to a change in nutrient supply or both remains to be investigated.

The resultant effect seems to be an upsetting of the balance of growth as seen by lower sap concentration and nitrogen content of the diseased leaves. If the disorder starts early, the yield is seriously affected, if it appears later, the lint quality is affected.

Soil deterioration during the crop-season seems to be the deciding factor favouring the disease. Suitable treatment of the soil to enable it to maintain its fertility at a high level throughout the cropping period (as shown by small plot tests) promises to overcome the malady.

We acknowledge with pleasure our indebtedness to Mr. F. K. Jackson, for his critical discussion and amendments in the draft. Our thanks are also due to Messrs. R. K. Aurangabadker and S. Ghosh for the various chemical analyses of which a few are included, and to Messrs. K. R. Joshi and Shamser Singh, Agricultural Officers at Jaipur and Bikaner respectively for their painstaking collection of local data and samples.

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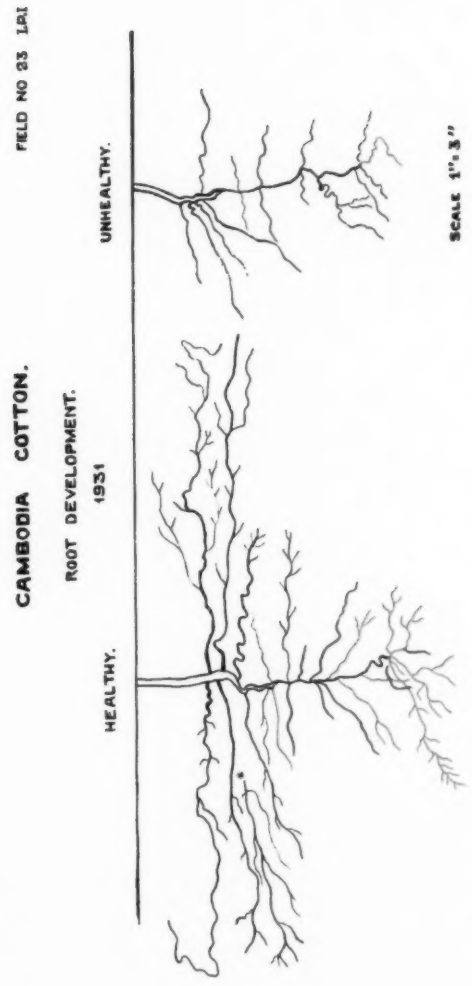
Coloured Plate No. XXVI (Leaf Roll and Red Leaf-Blight on Cambodia) will appear in Vol. IV, No. 2, Section B.


CAMBODIA COTTON.
ROOT DEVELOPMENT.
1931
FIELD NO 23 LRI
HEALTHY.
UNHEALTHY.
SCALE 1"=3"

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APPENDIX.

List of Disease Resistant Varieties of Some of the Important Indian Crops. (The information contained in the Table below was obtained through the courtesy of Directors of Agriculture in India.)

Variety or pure line	Complete immunity or low susceptibility	Name of disease	Tract or district in which it is found to be disease resistant	Remarks
1	2	3	4	5
		WHEAT (*Triticum vulgare*, Host)		
Pusa 114	Highly resistant	Rust diseases due to (*Puccinia graminis*, *P. triticina* and *P. glumarum*) and loose smut due to *Ustilago tritici*	Sind (not tried elsewhere on large scale)	
Pusa 80–5	do.	Rust diseases due to *Puccinia graminis*, *P. triticina* and *P. glumarum*	General	
Pusa 120	do.	do.	do.	
Pusa 165	do.	do.	do.	
Pusa 4	Resistant	do.	do.	
Pusa 12	do.	do.	do.	

Pusa 52	do.	do.	do.	
Pusa 101	Resistant	Rust diseases due to *Puccinia graminis*, *P. triticina* and *P. glumarum*.	General	
Pusa 111	do.	do.	do.	
C. 121	Complete immunity	Yellow rust *P. glumarum*.	Lyallpur (Punjab)	
C. 591	Low susceptibility	Yellow and black rust (*P. glumarum* and *P. graminis*).	do.	Hybrid line, has excellent amber coloured grains.
9 D	Almost complete immunity.	Loose smut (*Ustilago tritici*).	do.	Improved wheat for *barani* conditions.
8 B	Low susceptibility	Yellow rust (*Puccinia glumarum*).	do.	
T 11	do.	Black rust (*P. graminis*).	do.	
8 A	do.	Bunt (*Tilletia indica*).	North-West Frontier Province.	The varieties are given in descending order of susceptibility.
Pusa 4	do.	do.	do.	
C. 518	do.	do.	do.	
Pusa 52	do.	Rust diseases	North Bihar	

Variety or pure line	Complete immunity or low susceptibility	Name of disease	Tract or district in which it is found to be disease resistant	Remarks
1	2	3	4	5
Pusa 114	Low susceptibility	Rust diseases	Sind	
C. Ph. 47	do.	do.	North and Middle Sind	
Sind A. T. 38 ..	do.	do.	Northern Sind	
Sind H. S. W. III ..	do.	do.	Eastern Sind	
		PADDY (*Oryza sativa*)		
Pusa T. 18	Resistant	Piricularia (*P. oryzæ*)	Not tried on large scale.	Early variety.
Sathi	Completely immune	*Leptocoriza varicornis*	United Provinces	The immunity is due to the sheath cover protecting the ear.
Madras types.				
GEB. 24	Highly resistant	Paddy blast (*Piricularia oryzæ*).	Coimbatore, Adutturai and many other places where it has spread	Susceptibility reported from Coorg. Large supply of N_2 and consequent rank vegetative growth of plants reduces resistance.

	Completely immune	Foot-rot (*Fusarium moniliforme*).	Coimbatore, Maruteru, etc.	
	Low susceptibility	Stem-borer (*Schœnobius incertellus*).	Maruteru	Late sowing reduces resistance.
Co. 1	Highly resistant	Paddy-blast (*P. oryzæ*)	Coimbatore, Aduturai, etc.	Not so widely spread as GEB. 24 outside Coimbatore.
	do.	Foot rot (*F. moniliforme*).	Coimbatore, Maruteru	
Co. 4	Completely immune	Paddy blast (*P. oryzæ*)	Coimbatore and Aduturai	
Krishnakatakkulu ..	Low susceptibility	Foot-rot (*F. moniliforme*)	Maruteru and Coimbatore.	Godavari delta variety.
Akkulu	do.	do.	do.	do.
Nullarlu	do.	do.	do.	do.
Atragada	do.	do.	do.	do.
Vankisannam ..	do.	do.	do.	do.
Wateribune.. ..	do.	do.	do.	An American variety.
Aryan	Highly resistant	do.	do.	Malabar variety.

Variety or pure line	Complete immunity or low susceptibility	Name of disease	Tract or district in which it is found to be disease resistant	Remarks
1	2	3	4	5
		JOWAR (*Sorghum vulgare*)		
J. 301 and J. 8 ..	Low susceptibility	Red leaf	Punjab	In very humid years the resistance is lowered.
J. S. No. 21 ..	do.	Red leaf spot and smut.	South-Eastern Punjab	do.
Imphee and J. S. 20	Very low susceptibility.	do.	do.	
Sind Allakh and Sind Allakh X Torh hybrid No. 4, 54, 339, 109.	do.	Stem-borer	Sind	
		BARLEY (*Hordium vulgare*)		
Pusa T. 21	Resistant	Stripe disease (*Helminthosporium sativum*).	North Bihar Not tried elsewhere	
Plumage Archer ..	Complete immunity	Black and Yellow rusts.	North-West Frontier Province.	Introduced from England in 1934.

		OATS		

		OATS (*Avena sterilis*, L.)		
B. S. 1.	Resistant	Covered smut (*Ustilago kolleri*).	North Bihar Not tried elsewhere	
		LINSEED (*Linum ustitatissimum*)		
Pusa T. 12	Low susceptibility	Rust (*Melampsora lini*).	North Bihar	
Pusa T. 121 ..	do.	do.	do.	
Pusa T. 124 ..	do.	do.	do.	Mutant from T.12.
S. 6	Complete immunity	do.	South eastern Bihar	
P. 12	do.	do.	do.	
Hybrids 1150, 1193, 1206 and 1196.	do.	do.	United Provinces	With high seed and oil yield.
		GROUNDNUT (*Arachis hypogæa*)		
K. 17	Low susceptibility	Tikka leaf spot (*Cercospora* sp.).	Bihar and Orissa	
		COCONUT (*Cocos nucifera*)		
Philippine variety ..	do.	Shoot-rot (*Gleosporium* sp.).	West Coast—Madras	

Variety or pure line	Complete immunity susceptibility	Name of disease	Tract or district in which it is found to be disease resistant	Remarks
1	2	3	4	5
		SOYA BEANS (*Glycinea soja*)		
Pusa yellow ..	Low susceptibility	Root-rot in early stages and white ant in later stages.	Sind	
		SAFFLOWER (*Carthamus tinctorius*)		
Pusa types 9, 17, 18 and 19.	Complete immunity	Frost	Sakrand (Sind)	
Mixed Pusa—H. 68 and H. 12.	do.	do.	do.	
Dhollra I—Raichur	Low susceptibility	do.	do.	
		PIGEON PEA (*Cajanus indicus* S.)		
Pusa types 16, 41, 50, 51, 80 and 82.	Highly resistant	Wilt due to *Fusarium vasinfectum.*	North Bihar Not tried elsewhere	
Comilla, white, black, brown and crimson.	Low susceptibility	Wilt due to *F. udum* But.	Bengal	
S. 2 and S. 7 ..	do.	Wilt	North Bihar	

		GRAM (*Cicer arietinum*, L.)		
Karachi	Complete immunity	Wilt (*Fusarium* sp.)	Mouywa, Kyankse and Yamethin districts in Burma.	
Do.	Low susceptibility	do.	Central Provinces	
W. R. 18	98 per cent. resistant	do.	Dharwar (Bombay)	
Coimbatore types 416, 468 and 482.	Low susceptibility	do.	Coimbatore (Madras)	
		SUNNHEMP (*Crotolaria juncea*)		
D–IX	Complete immunity	Wilt (*Fusarium vasinfectum*).	Bombay	Homozygous; resistant under all conditions.
		JUTE		
D. 154	do.	Chlorosis	Bengal	
		COTTON (*Gossypium*)		
G. peruvianum varieties from South America, Verdav, Quebradinho and Moco.	Very low susceptibility.	Stem Weevil (*Pempheres affinis*	Coimbatore (Madras)	These are late varieties with bad opening and susceptible to Jassids.
G. hirsutum				
Cambodia: Co. 2 ..	Practically immune	Jassids	South and Central India and South Africa.	

Variety or pure line	Complete immunity or low susceptibillty	Name of disease	Tract or district in which it is found to be disease resistant	Remarks
1	2	3	4	5
Bengal, Cawnpore, Banila, Bengali, 85·10 (13), 158·16 (3), Garohill, Dacca muslin.	Low susceptibility	Wilt (*Fusarium vasinfectum*).	Bengal	
America, Cambodia K. A. I., 73·40 C, 7 (10), 41·30·9 (19) Akola Buri, Extra early Buri and 289F.	do.	do.	do.	
G. herbaceum				
Jayawant	92% resistant	do.	Bombay Karnatak	Soil temperature of 22–27° C., lowers resistance.
Broach Deshi—8 ..	Highly resistant; only shows root discolouration in 1 to 8% of plants.	do.	Broach District (Bombay)	do.
G. hirsutum				
Gadag I	60% resistant	Red leaf blight	Dharwar (Bombay)	
Buri	Complete immunity	Wilt (*Fusarium vasinfectum*).	Nagpur (C. P.)	
Verum, 262, 436 and	Low susceptibility	do.	C. P. and Berar	

Verum, 262, 436 and late verum.	Low susceptibility	do.	C. P. and Berar	
Roseum and Bani ..	do.	Anthracnose	do.	
		SUGARCANE		
Co. 205	Highly resistant	Mosaic	Coimbatore (Madras)	Reported to be susceptible at Pusa and other places.
Co. Nos. 214, 244, 314, 315, 316, 318, 335, 351, 355, 356, P.O.J. 2878.	"Immune"	do.	do.	Not reported to be susceptible from elsewhere.
Co. 214	do.	do.	Bihar and Orissa	
Co. 205, 210, 213, 281, 285. H. M. Nos. 320, 487, 544, 607–09.	Highly tolerant	do.	do.	
D. S. 1, 8, 19, 23–25 and 27–35.	Low susceptibility	do.	Bengal	
Thin varieties are more susceptible than thick ones.		Smut (*Ustilago sacchari*).	Coimbatore (Madras)	
Co. 205, 210 and 214	do.	do.	Bihar and Orissa and United Provinces.	
Co. 285	do.	do.	North-West Frontier Province and United Provinces.	

Variety or pure line	Complete immunity or low susceptibility	Name of disease	Tract or district in which it is found to be disease resistant	Remarks
1	2	3	4	5
Co. 290	Complete immunity	Smut (*Usilago sacchari*)	North-West Frontier Province and United Provinces.	
Co. 290	do.	Red rot (*Colletotrichum falcatum*).	do.	
Co. 285	Low susceptibility	do.	do.	
Co. 205, 214, 285 and 299	do.	do.	Bihar and Orissa	
Co. 205, 213, 214, 281, 285 and 326.	do.	Top rot (*Melanconium sacchari*).	do.	
Co. 214	Resistant	Insect attack (*Pyrilla*)	do.	
Co. 285	Low susceptibility	Stem-borer	do.	
		TOBACCO		
Adcock, cash, shade, Pusa type.	Resistant	Wilt (*Phytophthora parasitica*).	Coimbatore	Have shown resistance under laboratory conditions.

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